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PRESIDENTIAL ADDRESS

CONCERNING FUNGI INHABITING SOIL

By CHARLES G. C. CHESTERS

I well remember the impression which a conversation overheard in a certain botanical laboratory made upon my mind at the time when I finally decided to foray in search of mycological knowledge. Two friends were discussing certain mould cultures isolated from dilute suspensions of soil. One of these friends, who later became my much respected mentor in the field study of fungi, asserted forcibly, and with an emphasis of which he was a most effective master, that soil fungi as soil fungi did not exist and, further, that any attempt to identify a fungus in a test-tube was doomed to failure. It was against all the accepted tenets of mycological faith. Since, at that time, I was isolating fungi from small particles of soil plated out on agar these assertions shook me to the core. These isolations constituted an interesting pastime which appeared to be not devoid of possibilities. To have a senior colleague declaim in such a categorical manner made me pause and consider. In later conversations he expounded his views—that fungi were brought into the soil from above ground, that many so-called soil fungi were only temporarily resident in the soil, and that any fungi growing in that medium were in reality inhabiting particles of organic matter. Still, the urge to look below the surface stayed with me and at odd times drove me back to similar inspections.

The forays of this Society introduced me to methods of studying fungi inhabiting the surface of the earth, the bark and wood of fallen trees, and the waters of lakes and streams. At odd intervals, soil-inhabiting fungi were mentioned and, once in particular, a member of the Society quoted an American colleague as having stated that it was little use continuing to isolate moulds from the soil unless one knew that something was happening in that soil and unless one were isolating from that particular spot. It occurred to me then, and has constantly recurred to me since, that these words aptly describe precisely what a field mycologist does every time he collects a species or a group of species from a particular location. Experience has taught him to recognize some set of conditions in operation now, or the resultant of some past activities, which are associated with the appearance of the species. He is usually dealing with substrata of quite large size—pieces of material which can be picked up and handled. The fungus which they bear can be examined with a lens and, with its substratum, can be tucked away in basket, vasculum or envelope for further and more exact study. His collecting grounds include woods and meadows; orchards and arable fields—pleasant places, where he may wander and survey the prospect near or far of organic substrata on which the specimens of his species grow and flourish. Always his eyes are scanning the host or

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the substratum for these conditions which will signify to him the likely presence of what he seeks. The habitat is the resultant of many factors, chemical and physical, and it is seldom static. This changeful nature underlies the succession of species which is an everyday experience to the field mycologist and which frequently indicates to him the presence or the future prospect of particular species.

Turn now to the soil and examine a vertical surface cut downwards through a pasture turf or an arable field in crop. Consider the upper 6 in. or so of this soil profile and there you have the major part of the compound habitat in which collection and study of soil-inhabiting fungi must occur. This is the micro-field in which our forays must be conducted. Superficial layers of superimposed plant detritus of varying age and of varying degrees of recognizable structure give place to more compact debris, which becomes more highly diluted by mineral particles and gradually loses structural features until visible recognition becomes impossible, and the only sign of organic materials is the darker colour of some soil particles. Penetrating downwards through these successions of layers are the roots of plants, some of which are alive and glistening while others are dead skeletons of their former selves. Occasional large fragments of recognizable stem, leaf or root material occur as wanderers far below the surface layer, having been brought down by animals into the soil. Moist air fills the cavities between the living roots, plant debris and mineral particles. Such is our field of exploration. Can you visualize the similarities and the contrasts between this location for a foray and the location for one of our annual excursions in search of fungi? In broad general principles our usual hunting grounds represent magnified, mirror images of the soil—the debris of fallen trunks and branches match in space, if not in substance, the leaf and stem fragments at the surface of a meadow soil and the leaf and twig debris at the surface of a wood; the tree trunks, alive or dead, mirror the root systems below the ground; these detached branches, perched in a tree, which, because of their inaccessibility, may be most prized and are generally most unproductive of fungi, correspond to those larger fragments of unspecified origin below the soil surface. It is not intrinsically so different though the materials are less durable, their size is smaller and the 'atmosphere' in which they exist is less variable. But these differences are sufficient to introduce noteworthy complications. In place of more or less sizeable fructifications, the investigator in this field is faced with microscopic assemblages of sporangia or conidia, of oospores embedded in plant debris and of minute perithecia with their contained ascospores—if he is lucky. More often he encounters sterile mycelium, frequently of a most obdurate and intractable kind which no amount of persuasion will persuade to fruit. Hardly collectable specimens—certainly not to be initially studied with a hand lens, removed and packeted, but none the less fungi, aping in their saprophytism and their parasitism; in their comings and their goings; in their fruiting and their dispersal, many of their larger relatives above ground. While their study should be approached in just the same way as that of fungi above ground, if maximum knowledge is to be accumulated of their effective life in soil and of its implications to mankind, it is essential to realize that their identification is almost always a matter of difficulty, depending entirely on cultures established on sterile media.

How have we set out to investigate this field, what have we made of its problems, how have we studied its inhabitants? At the outset I want to make it perfectly clear that fungi are not the only tenants of this underworld just as they are not, thank goodness, the sole tenants of the world above ground. It is normally a well-balanced microcosm, but when unduly disturbed it has its storms and upheavals. Recent reviews by Burges (1939), Niethammer (1937) and Waksman (1944) have dealt with particular aspects of the distribution and the activities of soil-inhabiting fungi. I shall only deal here with limited aspects of the problem which appear to me to indicate future trends of development in this important section of mycology.

Sixty-two years ago Adametz (1886) initiated the study of soil-inhabiting fungi when he investigated the microflora developing in media sterilized in flasks and thereafter inoculated with soil, taken from the surface and from some depth in sandy and in loamy fields. From these flasks, inoculated in the open, he obtained eleven species of fungi. Koning, in partnership with Oudemans (1902), so improved the technique of isolation that his methods are worthy of more attention than they have been accorded. He inoculated plates of wort agar or wort gelatin with aqueous suspensions obtained by pulverizing fragments of vegetable matter extracted from soil in a given volume of water in a sterile crucible, and by diluting a portion of this suspension in sterile water. Some forty species were identified by Oudemans. To me this represents the first serious attempt to investigate the fungal content of soil, and it is the foundation upon which much recent work has been built. These investigations demonstrated the presence of moulds in soil of high organic content, particularly in relation to the organic matter, and indicated the appreciable variety of species which occurred in such conditions. Hagem (1908, 1910*a*, *b*) concentrated his attention on the Mucorales in Norwegian soils, and was the first person to plate out particles of soil on sterile media and to make transfers within a short period from the colonies arising from the inocula. In subsequent investigations the practice normally employed by bacteriologists for obtaining dilute suspensions was applied to the soils. Rivas (1910) was perhaps the first to introduce the method of collecting the soil sample by forcing a sterile tube into soil through a freshly exposed surface, and this method has been repeated by a great many investigators in soil mycology. C. N. Jensen (1912) employed a sterilized, tubular corer to obtain a cylindrical soil sample, due care being exercised to protect the sample from aerial contamination. He also reviewed previous records of fungi obtained from soil and presented a summary of the extent of our knowledge at that time. It is of significance that the study of fungi as organisms present in the soil had claimed few disciples as compared with the study of bacteria in the soil. In this science the method of progressive dilution and of plating soil suspensions had reached a very precise stage, and estimates of the numbers of bacterial units in known quantities of soil had become a matter of routine. Conn (1917), who was a bacteriologist, asserted that it was an open question whether, in fact, fungi lived and grew in the soil and suggested that isolations from soil might only reflect the presence of dormant spores. Waksman (1916) had already shown that, using particles of soil as inocula on agar plates, fungi could be isolated within twenty-four hours from the colonies produced by incubation at about 30° C. He further compared the rate of growth from inocula of hyphae and from inocula of spores of the same species, and demonstrated that the former produced the larger colonies in an initial limited time period and therefore deduced that the earliest visible growth came from mycelium already present in the soil. In further studies, employing a technique for staining smears of soil, Conn (1922) obtained microscopic evidence for the presence of mycelium in soils of very diverse natures.

Meanwhile, quantitative data were accumulating regarding the so-called numbers of fungi in the soil (Waksman, 1922; Brierley, Jewson & Brierley, 1927). Most of these investigations depended upon essentially bacteriological methods which provided dilutions of known concentration relative to a given weight of soil. That these dilutions contained spores as well as fragments of mycelium was recognized (McLennan, 1928). The accumulation of this knowledge represented, and still represents, a phase in the study of soil-inhabiting fungi. In all biological processes, the investigator is faced with the evaluation of his observations in terms of units which permit comparison between sets of his data and those of other workers in the same field. Although a great deal of labour has been expended on the estimation of numbers of fungi inhabiting the soil, we are forced to recognize that these labours have been successful only in part. So long as we recognize the fact that, apart from the excellency or otherwise of the method of estimation, we are dealing in such studies with a limited and frequently highly specialized and highly fecund section of the fungal population, then we are justified in accepting the results. It must be clearly understood that, quite apart from the higher Basidiomycetes, such frequent inhabitants of the soil as species of *Pythium*, species of *Mortierella* and a galaxy of the darker Hyphomycetes are seldom, if ever, reflected in their true relationships in such studies. Brierley *et al.* (1927) made an exhaustive study of certain of the factors which influence the results of plate-counts of fungi obtained from soil dilutions and Brierley (1923, p. 123) had already pointed out certain factors which must be considered in evaluating such estimations. Not the least of these is the wide differences in the vegetative structure and in the spore-producing apparatus of fungal species which are both inevitably reflected in the degree of separation of the units in such dilution plates. Local and abundant sporulation influences the estimate of fungal numbers even in the largest and most random of samples which can be handled effectively under stringent aseptic technique. But despite these and other limitations it is quite clear that maximum population is attained close to the soil surface and that the population decreases with increase in depth. One of the potent factors in operation is the decrease in effective organic substrata on which the fungi can live, while other factors include aeration, moisture, temperature and perhaps available soluble materials not related to the organic pabulum (H. L. Jensen, 1934). Lateral differences in fungal content are also experienced and are, in the main, exactly what has been experienced by any field mycologist as the natural state of affairs. It is very little use quoting precise examples of statements of the number of fungi per gram of soil because such figures, interesting and instructive as they may be, and I would not suggest otherwise, appear to be a compound of what is, what was and what may be. It is very difficult to visualize how this position may be remedied until a technique is to hand which permits easy and rapid visual examination and identification of these fungi which are growing in that same fraction of soil which is later diluted and plated. Circumstantial evidence has been obtained, however, and if sufficient data are accumulated it should lead to enlightened views on this subject.

Up to 1930, most of the investigations dealing with soil-inhabiting fungi were based on essentially bacteriological methods involving dilution of soil suspensions. The total sample was shaken up in water and that part which became free and remained suspended was introduced into agar and incubated. It was possible to give a quantitative statement of the number of fungal units isolated from a known weight of soil, always supposing each growing unit represented one original soil unit. Estimates of the numbers of fungi were obtained from soils differing in acidity and alkalinity; in organic matter of varying carbon content; in the level of nitrate, ammonium, phosphate; in moisture content; in the kind of cover vegetation and in many other respects. This kind of information indicated that viable fungal units can be extracted in no small measure from fertile soils containing organic materials, that the figures obtained varied with the richness of the soil in organic matter of different kinds, and that apparently the species of fungi did not differ much from soil to soil within a region, except in so far as species of *Penicillium* were commoner in temperate soils, species of *Aspergillus* in tropical soils, species of the Mucorales in woodland and cultivated soils and the general run of pathogenic species were only to be obtained in quantity where suitable hosts grew or where conditions favoured their continued existence. Valuable information was accumulated on diverse topics, but only a very indistinct picture of the fungus at work had been obtained; in fact, the working fungus was only really visible between the upper and the lower parts of a Petri dish or within the cylindrical wall of a culture tube. But, in 1928, Rossi devised one of those simple methods of biological experimentation which act as the trigger to set all sorts of reactions in motion. He pressed clean microscope slides against a freshly cut surface of soil and, after fixing this by heating, stained the preparation to display bacteria, fungi, and other members of the soil population in place relative to one another and to the soil particles. This was indeed an advance—almost like looking at the private life of soil organisms in a cinema 'still'. Cholodny (1930) modified this method by fixing the slide against the fresh soil surface with the loose soil removed to expose the surface, and by removing the slide for fixation and staining after a period of incubation. Conn (1932) applied this method to soil in vessels so that the flora in different soils could be studied or the effect of different degrees of moisture and of different levels of fertilizer additions could be determined. He suggested that the colony formation of pure cultures in sterile soil could likewise be examined. The Cholodny technique was subsequently used in many soil studies (Demeter & Mossel, 1933; Garassini, 1939; H. L. Jensen, 1934; Timonin, 1940*b*; Verplanke, 1932; Ziemielka, 1935). It must be mentioned here, however, that reflexion on the scope and accuracy of this method raises doubt whether in fact it does picture the soil flora as it lives. True, no nutrient is added to the soil, but the glass surface allows moisture accumulation, permits local proliferation of bacteria and may even act as a surface for the transport and passage of fungal hyphae which is not normally present. It certainly disturbs the aeration of the soil. Nevertheless it has been modified and applied as a quantitative method by H. L. Jensen (1935), who obtained interesting indices of the relative richness of different soils in fungal mycelium. A further and a rather different method of estimation of the fungal mycelium in soil has been devised by Jones & Mollison (1948) in which small quantities of soil are pulverized in a small volume of water and the suspension so obtained is diluted with agar and from this dilution films of constant thickness are prepared, dried and stained.

None of these methods examines the living fungal population in situ. This has only been possible since the design and perfection of a system which allows vertical illumination of an object under examination, by high microscopic magnifications. Such vertical illumination was applied to soil studies by Kubiena (1932, 1935), and has resulted in the very interesting discovery of active growth of certain species which have not usually appeared in dilution plates. This method has definite promise, especially as it is possible to remove inocula from species seen growing in the soil-cavities using micro-instruments and to isolate from such inocula. I have adapted the Leitz Ultrapak for use in this method, but it must be clearly pointed out, however, that since the technique is laborious and since the results are not always of an equally high quality this method would appear to be better applied for the moment to limited and particular studies. It is decidedly not a routine method, but in the hands of a trained experimenter should yield interesting data not otherwise obtainable. The use of tubes with capillary orifices in the lower parts of the tubes and filled with sterile medium (either a nutrient agar or a mixture of soil and organic material) which are immersed in soil for an incubation period has already been described (Chesters, 1940, 1948). The species of fungi which are isolated from such tubes usually possess a widely spreading mycelium and grow actively in the soil. Although this method does not permit visual inspection of the soil for active mycelium there is little doubt that living hyphae must be present in the soil adjacent to the tube before the species can be isolated.

Taxonomic studies of species isolated from soil are to be found in many individual papers and not a few monographs. Species of the Mucorales have received much attention (Campbell, 1938; Dixon-Stewart, 1932; Hagem, 1908*a*, *b*, 1910; Jensen, C. N., 1912; Lendner, 1908; Niethammer, 1935; Pispek, 1929), as have certain limited genera of the Fungi Imperfecti and a few Ascomycetes. Butler (1907) initiated a fresh approach specially profitable with species of *Pythium* and allied genera, and his methods of isolating such fungi from bait floating in water covering the experimental soil have been modified and applied by several investigators with most interesting results (Matthews, 1931; Middleton, 1943; Sparrow, 1943). From such studies, the range of species and the taxonomy of these species has been very fully considered. Gilman and Abbott (1927) and more recently Gilman (1945) have brought together most of the taxonomic literature on soil-inhabiting fungi in book form. It is still essential that more detailed studies should be undertaken, and I see little hope of stable and enduring work in this aspect of soil-inhabiting fungi until full documentation of isolated species by means of adequate descriptions, drawings, slides and dried culture specimens becomes a matter of routine. There is still an immense field to be investigated and many discoveries to be made.

Surveying the present state of our accumulated knowledge of the soil-inhabiting fungi, as discovered by the methods of investigation I have outlined, we are faced with the fact that most of this knowledge concerns species or genera in relation to gross habitat factors as reflected by their geographical range (Kursanoff & Stiklyar, 1938; Niethammer, 1935, 1937; Rayllo, 1928). Fungi have been isolated and described from, and their numbers have been estimated from, cultivated soils (Chand, 1937; Chaudhuri & Sachar, 1934; Ghatak & Roy, 1939; Jasevoli, 1924; H. L. Jensen, 1931 *a*, *b*, 1934; Singh, 1937 *a*; Ziling, 1932), from forest soils (Ellis, 1940; Paine, 1927; Pistor, 1930; Svinhufvud, 1937), from soils with high salinity (Bayliss-Elliot, 1930; Killian & Feher, 1935; Sabet, 1935) or with high organic matter content (Verona, 1934), from below particular kinds of cover vegetation (Deyl, 1938; Le Clerg, 1931), from cultivated as compared with natural soils (Bisby, James & Timonin, 1933; Dixon, 1928; Janke & Holzer, 1929; Ma, 1933 *a*; Samoutzevitch, 1927), from particular soils at differing seasons of the year (Dixon, 1928; Ma, 1933 *b*; Svinhufvud, 1937); from different depths in a particular soil, the depth being but seldom related to the soil horizons (Bisby, Timonin & James, 1935; Deyl, 1938; Jasevoli, 1924; Le Clerg & Smith, 1928; Paine, 1927; Swift, 1929; Todd, 1932). The relationships of fungi in the soil to organic matter (Thom & Morrow, 1937; Vandecaveye & Katznelson, 1940), to moisture and temperature, to soil fertility in relation to crop growth (Singh, 1937 *b*) have been discussed in several of the papers mentioned, but very little information can be obtained of exactly where in the vast expanse of the soil the fungi are actively growing—what precisely constitutes the individual habitat. The broad backcloth had been sufficiently painted in by 1916 for Waksman to be able to indicate the world-wide distribution of many species of fungi—some of them apparently not known from any other source. He could confidently portray a group of soil inhabitants remaining relatively constant in their occurrence in world-wide soils, but showing a replacement of species of *Penicillium* by species of *Aspergillus* in the warmer regions of the world. At times these local inhabitants were increased or modified by varying species of the common genera, and at all times, alien species, not of this select group and evanescent in their stay within the soil, constituted the invader species. This idea of soil inhabitants and soil invaders is good just so long as you are content to work with gross isolation techniques, but one wonders if it will continue to hold when, as one hopes, soil will be subject to detailed collecting excursions. It is true that Reinking & Manns (1933) related these concepts to species of *Fusarium* in Central America and demonstrated for one genus that there are species so specialized in their parasitism that they cannot long survive as living units of the soil population apart from their host material, living or recently dead. Garrett (1938) very precisely defined these terms in relation to fungi attacking plant roots and has demonstrated the very real value of the concept in this field.

Let us examine the soil inhabitants for a few moments, but, before we do so, it is worth while remembering that even in 1947 the backcloth has a wide expanse unpainted. Basidiomycetes do not fully occupy even a corner of it, yet in the field they are obviously one of the very old inhabitants. Their isolation is a matter of getting the right technique—which so far has eluded us. In a survey of the lists of fungi isolated from soil it is still as true to-day as it was in 1916 that species of *Mucor*, *Penicillium*, *Trichoderma*, and *Aspergillus* predominate, and are closely followed by species of *Rhizopus*, *Zygorhynchus*, *Fusarium*, *Cephalosporium*, *Cladosporium* and *Verticillium*. When we attempt to generalize regarding the fungi isolated from particular vegetational units (e.g. forest, grassland, arable land) there is much contradiction and uncertainty as to the frequency of even the commoner species. It can serve little purpose to attempt a review of these here because the reasons for this variation are many and not the least of them is the method of isolation employed. It is worth pointing out that in many forest soils species of the Mucoraceae appear with great frequency and Hagem's (1910*a*, *b*) concept of a community of species of *Mucor* has been abundantly confirmed (Niethammer, 1935; Pistor, 1930). The dominant species is usually *M. rammanianus* in association with other species of this or related genera which vary from place to place. In general terms it may be said that species of *Mucor* and related genera are more frequent in forest soils (Paine, 1927) than in grassland soils where, particularly on acid soil types, *Penicillium* and *Trichoderma* are commonly reported. This relationship of species of *Mucor* with the forests is of some interest and may be not unconnected with the inability of these fungi to hydrolyse cellulose and with their ability to decompose protein (Abbott, 1923; Martin, Anderson & Goates, 1942). It is precisely in such situations that high protein content may be expected in the annual carpet of leaves deposited without previous undue destruction.

As well as the higher Basidiomycetes there are other striking omissions from the components of the fungal flora usually reported from soil. Species of *Pythium* may be considered. With the possible exception of Meredith (1940), and of the investigators mentioned above (p. 203), few workers in this field record species of the genus and yet they are commonly present in natural turf soils and are specially prolific in cultivated soils under vegetable crops. *Pythium ultimum* in an active state (Chesters, 1948) can be isolated from such soils during all but the driest seasons of the year and it is almost true to say that *P. intermedium* is one of the most frequent of soil-inhabiting fungi. But all species of this genus prefer maize extract agar as their isolation substrate. Again *Rhizoctonia solani* (*Corticum vagum*) is not often recorded from pasture and from cultivated soils, yet its typical mycelium may be observed associated with plant debris in almost all soil rich in organic matter. It grows freely from debris on to agar, but will not often appear in dilution plates. Many other instances could be cited of similar omissions, and before leaving this matter I would draw your attention to the infrequent and irregular reports of species of the darker Hyphomycetes. Such fungi are present on plant debris in the upper layers of the soil and play no small part in the decomposition of structural material. They may perhaps be invaders, but some at least can be regularly obtained from precisely similar locations in the soil (e.g. *Helminthosporium biforme*, Mason & Hughes, in appendix to Chesters, 1948). It is possible that their absence is due to the difficulty of germinating detached spores or mycelial fragments such as would normally be present in suspensions. These difficulties are well known to those who attempt to grow species of the Dematiaceae collected above ground.

I should like to return for a few moments to the soil profile. Have we given enough thought to what goes on above the soil in relation to what we are striving to examine within the soil? The basic nutriment of fungi living in the soil ultimately derives from organic matter of plant or of animal origin—it may be the direct pabulum on which they live or it may supply the solutions on which free-living hyphae, if there are such, depend, and it almost certainly plays a part in the initial germination stages of spores in the soil. The plant fraction is obtained either from the aerial shoot systems or from the root systems. The latter, on death, and in so far as saprophytic fungi are concerned, are directly vulnerable to the fungus mycelium or to the fungus spores already present in the soil. The former present a very different state of affairs. The basal parts of the shoot system in contact with soil may be directly available to soil-inhabiting fungi but the distal parts are by no means thus exposed. They, on the contrary, are 'worked over' by weak parasitic and by saprophytic species; by a whole galaxy of Fungi Imperfecti, of Ascomycetes and even a few Hymenomycetes and they arrive at the soil surface very much depleted of their nutritional possibilities—they have lost much, but they have gained much which was foreign to their original make-up. Perhaps the one important exception to this state exists in deciduous trees and shrubs where the leaves reach the soil level little changed through prior fungal attack. Elsewhere air-borne saprophytes or weak parasites have utilized the easily available proteins and carbohydrates before stem or leaf reaches the soil and the continued growth of some of these species frequently depends on just this change of nutrition. From the point of view of the soil-inhabiting fungi such pretreatment means that cellulose complexes and less easily soluble nitrogen compounds, not forgetting the protoplasm and fungal cellulose of the mycelium of these vanguard fungi, is all that is left to the lot of the soil inhabitants. It is not to be wondered at that many of these actively hydrolyse cellulose (Dubos, 1928; Jensen, H. L., 1931 *b*; Karnicka & Ziemiecka, 1935; Skinner, 1925; Vandecaveye, 1935); their power in this respect is linked with the availability of nitrogen, and Waksman has indicated that the ratio of cellulose decomposed to nitrogen utilized is in the region of 30:1 and that this ratio is relatively constant both in vitro and in field experiments. Since the ability to decompose cellulosic materials is closely linked with the availability of nitrogen, it is just at this point that the mucoraceous types are so important in soil as they are so predominantly the forms which liberate this nitrogen. To accomplish this effectively, not only proteins but also easily available carbohydrates are necessary—in nature they can be obtained from fresh or relatively fresh plant debris reaching the soil. If this argument is sound, it would explain why the species listed in diverse soil studies are confined to Mucoraceae and cellulose-decomposing Fungi Imperfecti and to such Ascomycetes as *Chaetomium globosum* and its allies. The occasional species of such lists may be derived from spores detached from mycelium of Fungi Imperfecti which is living in organic debris or spores which have been carried down from above. In the upper horizons of the soil such fungus-bearing debris occurs in quantity, and it is here that the filtering action of the soil is so manifest. While most studies of soil fungi have been conducted in these horizons the actual surface debris has frequently been carefully removed. I am certain that soil investigations in future must commence by examining this surface litter and must work downwards into the lower horizons. I agree that the task is difficult, that its danger lies in the inability to surface sterilize effectively, by currently accepted methods, portions of plant remains, and that selection in sampling must be the rule. Walker (1941) has already indicated that various surface sterilization methods affect the isolation results in no small degree. However, I am fully convinced that prolonged washing with renewed volumes of sterile detergent solution is probably just as effective in removing unwanted spores and other contaminants as is any treatment designed to kill chance spores. Combined with a careful scrutiny of the time of appearance of mycelial growths and with a careful selection of isolating media—not all of which need be agar—much useful information regarding the location of individual species, the grouping of species relative to units of material, the seasonal rise and fall of species during changes in the soil and the succession of individuals or groups of individuals, will be obtained. It is my view that through such studies we shall ultimately come upon the missing link in soil mycology—the elusive higher Basidiomycetes.

Within the more mineral portions of the soil similar considerations must be borne in mind. Moribund or dead roots reflect the same cycle of nutritional changes as the stem and leaf material, but the volume of nutrients is much reduced. A similar sequence appears to exist; mucoraceous types act as early colonists and are replaced by cellulose-destroying Hyphomycetes. The study of succession on root debris is probably one of the easier problems, as such debris can be effectively washed and yields results of some interest even on agar. Several investigators have noted the presence of species of *Mortierella* in the soil, and Bisby *et al.* (1935) go so far as to state that, in the Canadian soil profiles studied by them, species of this genus proved to be the most abundant phycomycete. From the systematic accounts of the genus it is well established that many species occur on decaying debris on the soil surface. We have isolated a considerable number of strains of *Mortierella* from root debris, but their taxonomic treatment is a matter of some difficulty. It would appear, however, that there are relatively few species concerned. With experience in recognizing certain mycelial characteristics, it is possible to pick out strands of undoubted *Mortierella* hyphae extending from root debris into the soil and even to cultivate such strands on agar. There is reasonable certainty that some species of *Mortierella* occur on root debris as a regular habitat. Certain hyphae passing out from root fragments form vesicles in close proximity to the root tissue and such hyphae yield very fine, thread-like and sterile mycelium in agar culture. Whether these are also, in fact, species of *Mortierella* is open to doubt and one cannot help reflecting in this connexion on the observations of Butler (1939) regarding the arbuscular types of mycorrhizal fungi in plant roots.

To summarize what has been said regarding the examination of soils carrying natural vegetation; much of the past work deals with mass isolation from particular horizons in the soil; the source of cultured species is various—mycelium, freshly produced spores and dormant spores all participate; biochemical studies with such fungi indicate broad lines of succession related to the carbon-nitrogen requirements. It would appear that in the future, a new and profitable line of attack should be the consideration of the biology of species in relation to the plant debris on which they grow, followed by attempts to examine and interpret the micro-habitats of the fungi concerned.

Conditions are less stable in cultivated soils—the annual act of cultivation and the repeated husbandry to which they are subject, causes not only physical changes in structure, but also great variation in the type and the condition of the organic material added to the soil, and in the nitrogen and general salt content of the soil. It is evident that the addition of any form of green vegetation immediately stimulates the local development of fungal mycelium and that mucoraceous species in particular respond to this stimulus, when high protein content is concerned. Such localized and intense activity in a medium of relatively high nutrient value should bring into play the fullest possible effects of antagonism consistent with the food supply, and it has been repeatedly emphasized that antagonism and antibiosis are only really effective under such conditions—but a more detailed study of such phenomena is urgently necessary.

In arable soils the development of a crumb structure is of vital importance from the point of view of the physico-chemical and of the biological aspects (Erikson, 1947). It is here that the soil fungi play a most important part. Mycelium passing through the soil helps to bind the mineral fractions into aggregates (Waksman & Martin, 1939) which tend to resist leaching effects of percolating water, and which improve the aeration of the soil and the ease of root traverse through the soil. Some interesting investigations on this aspect have been reported. The actual degree of stability of the crumb structure appears to be influenced to a varying degree by different moulds, when these are actively growing in the soil. Under definite experimental conditions Martin & Anderson (1942) have shown that, in silt and in sandy loam, the following series of increasing efficiency could be determined in relation to water stability of the aggregates:

Silt loam	Sandy loam
Rhizopus nigricans	*Rhizopus nigricans*
Penicillium glaucum	*Penicillium glaucum*
Mucor rouxi	*Aspergillus minutis*
Aspergillus minutis	*Mucor rouxi*
Cladosporium	*Cladosporium*

They point out that the order of effectiveness in producing water-stable aggregates approximately follows the successional appearance of these fungi during decomposition: *Rhizopus*, *Mucor*, *Penicillium*, *Aspergillus* and *Cladosporium*. Kanivets (1939) has demonstrated that aggregation at foci which develop in relation to micro-organisms in rhizosphere soil may be three or four times as great as aggregation in surrounding soil. *Trichoderma lignorum* appears to be particularly common in such aggregates, and artificial inoculation of soil with this species increased soil aggregation in the rhizospheres of cereals, pulses and root crops (Kanivets, Chariton & Tultshinskaya, 1940). Aggregation foci contained more free nitrogen, phosphorus and potassium than neighbouring soil (Kanivets, 1939). It would appear, therefore, that besides acting as centres for the development of crumb structure such local mycelial activity increases the available mineral constituents of the soil.

Study of the succession of fungal species, relative to plant debris in arable soils has an obvious bearing in relation to the species which are suspected of existing in a prolonged saprophytic phase in the soil. Such investigations may prove of particular interest with respect to species of *Phytophthora*. Although certain of these have been shown to be capable of living saprophytically in steamed soil (de Bruyn, 1922) there is no very clear evidence for or against the persistence of such mycelia in normal soil. I believe that the study of the fungal flora of plant debris in arable soil as it passes into ley may be of much interest and value in relation to the carry-over of specific pathogenic fungi from one crop to its next appearance in a rotation.

The fungal population of arable and natural soils has another important facet which requires much more extensive and intensive study than it has yet been afforded. Sachs and Knop in the later half of last century demonstrated that roots excrete materials into the external medium as well as absorbing salts from this medium. This encouraged Hiltner, in 1904, to suggest that the soil round roots should provide a good growing medium for micro-organisms, and to prove that micro-organisms were more numerous there than at other points in the soil. He called that area of soil which surrounded a root and received root exudates, the rhizosphere. The obvious importance of what is happening immediately external to the root tissues and to the root hairs need not be emphasized—but it should be realised that two very different sets of problems are inter-related at this point: root action on the soil population and soil population effect directly on the root as the absorbing organ of the plant. Starkey (1929) initiated the modern study of the rhizosphere population and demonstrated that for adult plants the fungal fraction of the soil population was less affected by the presence of living roots than was either the bacterial or the actinomycete population, but that all three were more numerous in the rhizosphere than in the 'open' soil. This work was carried out by dilution-plating methods and by virtue of that fact suffered initial inaccuracies. He demonstrated also that the maximum effect was evident in late stages of growth or during flowering and that to a certain extent plants of different species did not stimulate the soil micro-flora to the same extent. While the difference in estimated fungal population between rhizosphere soil and soil remote from roots was found to be not fully significant, the difference between the estimated fungal population on the surface tissues of roots and in the surrounding soil was marked (Starkey, 1931*a*; Thom & Humfeld, 1932). It would appear that the soil type has some influence on the degree of difference exhibited between the rhizosphere population and the population of open soil, and Adati (1939) has shown that the difference is greater in sand than in soil rich in humus. This result would support the theory that root excretions act as growth stimulants, because these are more likely to be potent in soils poor in other forms of nutriment.

The introduction of the Rossi-Cholodny slide technique provided a further method of examining and of estimating the population of the rhizosphere. By burying slides in relation to growing roots Starkey (1938) demonstrated the presence of fungal hyphae close to root hairs and to small roots. It was clear that while both living and dead roots bore such fungal associates, they were more numerous on the latter, where hyphae and spores were seen. Infection of living roots was also observed. In general, the hyphae within the rhizosphere of living roots did not sporulate, but in the open soil sporulation was observed on mycelium associated with minute particles of organic matter. Starkey comments on the occurrence of bacteria on the surface of living fungal hyphae, forming either diffuse patches over the wall or local aggregations. More numerous bacterial accumulations were evident on dead mycelium. That the bacterial colonies on living hyphae are probably related to the additional soluble materials in this region is highly possible. That the increases in bacterial numbers in rhizospheres may in part reflect the stimulation of bacterial growth following the death of fungal mycelium must not be overlooked. Timonin (1940 *a*) concludes that in the presence of young roots bacterial development is enhanced as compared with fungal development, and makes the very pertinent remark that this stimulation is probably in the mycelial condition and that free sporulation occurs more readily in open soil—perhaps in relation to the lower moisture content. Timonin has demonstrated the interesting fact that the rhizosphere population of individuals of the same variety of a flowering species exhibited little change when the soils in which the plants were growing had been subject to varying fertilizer treatments which themselves resulted in differences in crop yields. This is at variance with observations elsewhere in open soils.

There, addition of organic and of inorganic fertilizers stimulates the development of the soil micro-flora, including the soil fungi. It appears, therefore, that so far as the rhizosphere is concerned, the type of crop is more important than the fertilizer treatment. This may be interpreted as indicating the essential relationship of the rhizosphere population to the total metabolites of the region. In this connexion it is interesting to realize that Timonin (1940 *b*; cf. West & Lochhead, 1940) found the population of the rhizosphere of a flax susceptible to wilt to be higher than that of a flax resistant to wilt, and a similar relationship was demonstrated in varieties of tobacco, some susceptible and some resistant to black root rot. This confirms the similar reports of Thom and Humfeld (1932).

During earlier studies of soil populations in Manitoba, Timonin (1935) had demonstrated higher counts from soils below the wilting-point for flowering plants than from moist soils easily supporting vegetation, and in his studies on rhizosphere populations he drew attention to the fact that in controlled experiments rhizosphere populations were higher in soils of low moisture content. His explanation of this is worthy of notice, as he points out that the soil solution is more likely to be concentrated in soil at 30% of its total moisture holding than in soil at 60% and that the Cholodny slide technique demonstrated increased root-hair development in the drier soil. This larger absorbing surface, besides releasing additional nutriment by death of the older root hairs, is likely to increase the accumulation of the excreted materials into the rhizosphere and hence stimulate microbial development. Even in almost sterile, arid soils a strong rhizosphere population exists (Sabinin & Minina, 1932).

This important aspect of soil mycology must necessarily expand in future years when new methods of attack are devised and when much more is known about the physiology of the soil-inhabiting fungi. So far, the attack has been aimed at estimating the quantity of the total rhizosphere population and of the fraction of it which can be cultivated on agar plates; at inspecting small areas of this rhizosphere population as it adheres to slide traps inserted to apprehend it; and at demonstrating the effect of the growing plant roots on these aspects of the rhizosphere. Very little has been written of the effect of the rhizosphere population or its fungal fraction upon the normal healthy flowering plant. I am well aware of the extensive literature relating to the mycorrhizal habit and of the voluminous researches on the intimate relationships between root-attacking fungi, the soil micro-flora and the host root, but this field is beyond the path I have chosen to follow for the moment. A study of the part played by the rhizosphere in determining the soil solution available to the normal plant root is of much importance, and already studies are indicating that the availability of phosphates to the plant root may owe much to the action of the fungal portion of the rhizosphere. The production of nitrogenous and carbohydrate units by the soil fungi associated with the rhizosphere and their relationship to plant growth is likely to prove of interest, and it may well be that the flowering plant is shown to be far more heterotrophic than has been suspected, when a more complete analysis of the role of the soil microflora—and not the least, of the fungal part of that flora—has been made.

Thom and Humfeld (1932) have pointed out that the rhizosphere of maize roots gave equally high fungal counts in acid or in alkaline soils, and have inferred that the lack of significant variation of these numbers is due to the fact that the root zone carries its own pH value irrespective of the soil through which it passes, and that this zone is in the region between pH 6 and 7·5. The fungal population of such rhizospheres consisted predominantly of species of *Trichoderma* in acid soil and of *Penicillium luteum* and other species of this genus in alkaline soil. Melin (1934) has shown that roots secrete phosphatides and that these stimulate the development of soil-inhabiting fungi. It may well be (as D'Aeth has suggested, 1939) that this is a further potent factor in the relationship of the rhizosphere fungi and the plant roots. Jahn (1934) includes what are usually considered as rhizosphere fungi in his group of peritrophic mycorrhiza, stating that although they do not penetrate the root tissues they have a symbiotic function. He points out that Friesleben (1934) had already shown for *Vaccinium* that the addition of *Penicillium glaucum* (*sic*) to pure culture seedlings of the host produced a stimulation of the higher plant of similar order to that of a proper mycorrhizal partner. Jahn believes that the valuable contribution of the peritrophic fungi lies in the fact that they develop a zone of high acidity round their associated roots, and that such species as *Mucor racemosus* and *M. rammanianus* have strains which are confined to these root zones. According to him the characteristic fungi in such locations are species of *Penicillium* and *Mucor*. In later studies, Jahn (1936) expands this conception to all classes of mycorrhizal fungi (endo-, ecto-, and peritrophic). He believes their value lies in their ability to render available the bound salts of the soil by virtue of the production of an acid medium round the roots. It is probable that, particularly in relation to phosphorus availability (Abbott, 1923), this aspect of the rhizosphere will prove to be of great importance.

I should like to close by indicating the lines which appear to me to hold promise of future developments in the study of soil-inhabiting fungi. Foremost of these is an investigation of the crude organic matter at and immediately within the soil surface—I am certain that it is within this zone that the secret of the higher Basidiomycetes will be uncovered. I am equally certain that this can only be adequately pursued alongside pure culture studies of isolations from the sporophores of named species growing at the point of investigation.

Investigation of the space relationship of mycelium in the soil with reference to organic debris may lead us to modify the very useful conception of inhabitant and invader species to a concept of local and wide colonizers, and discover to us that the inhabitant flora consists of many more members which are constant in soil, but which, heretofore, have not been able to compete at isolation with the wide colonizers such as species of *Mucor* and their allies.

Development of methods of investigating the immediate surface of contact between soil and root must take place, and a united endeavour between the mycologist, the plant pathologist and the plant physiologist in this field is long overdue and of the utmost importance in understanding plant economy.

Elucidation of the natural effects of antibiosis, if such exist, of its whys and wherefores in the day-to-day life of the soil population must be seriously undertaken alongside the quite different and equally vital study of antibiotic action in human and animal affairs.

Work on the physiology of soil fungi, on their relationship to other members of the soil population, on their vital place in the physics of arable soils and on their economic effects in crop production are all ultimately based not only on relative quantities at work but also, and, I would plead, more cogently, on the precise individuals operating at one time. This knowledge cannot come without detailed and painstaking study of the taxonomy of soil-inhabiting fungi. This, in turn, is a matter for pure culture investigation in vitro, if not necessarily in agar-agar, and it may frequently require a deep appreciation of the intimate physiology of the species in question, as the excellent investigations of Melin and his school have demonstrated. The taxonomist working on soil fungi not only must be prepared to identify his species in a culture tube, without knowing what he put into it, but also must be capable of persuading the species to deliver up the criteria by which it may be known—in other words, must be capable of making it spore. New methods and new outlooks are necessary to study this fundamental field in which the fungus plays a vital part in the medium from which the essences of life in this world derive.

I wish to conclude by quoting from R. L. Stevenson a passage whose wide application to botany was first brought to my notice as an undergraduate student, and whose immediate application to the investigation of fungi inhabiting soil is patent: 'For to travel hopefully is a better thing than to arrive, and the true success is to labour.'

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A STUDY OF VIOLET ROOT ROT

II. EFFECT OF SUBSTRATUM ON SURVIVAL OF *HELICOBASIDIUM PURPUREUM* COLONIES IN THE SOIL

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It has been assumed, though so far without experimental demonstration, that sclerotia of *Helicobasidium purpureum* Pat. are responsible for prolonged survival of this fungus in the soil from one host crop to the next (Hull & Wilson, 1946). The sclerotia have been described by Duhamel (1728), Duggar (1915), Faris (1921), Hull and Wilson (1946) and Garrett (1946*a*). In the present paper are described some experiments on the survival of colonies of *H. purpureum* on different agar substrata, when buried in jars of soil in the laboratory. This work was planned as a preliminary to a projected study of the production of sclerotia on the host plant, and of their survival in the soil. Although this work is incomplete, circumstances prevented its continuation, and so the results are presented.

Method

Sclerotia can be observed in pure culture on suitable nutrient agars, and, when mature, can be picked off the plates for purposes of investigation; for work on a large scale, however, this method has serious disadvantages. Sclerotia are formed at different distances from the centre of the colony, and to this difference in position is to be added a great variation in size of individual sclerotia. Individual sclerotia, moreover, may fuse to form compound sclerotial masses, and it is difficult to detach sclerotia cleanly and without wounding from the substrate. It was therefore decided to use, not individual sclerotia, but individual small colonies of the fungus in this preliminary study of survival. Briefly, the method consisted in the inoculation of agar plates (9 cm. diameter) at ten equidistant points, 14 mm. from each other and 9 mm. from the margin of the plate. Inoculum disks, of diameter 4 mm., were cut out marginally from colonies on double strength meat-malt agar (for composition, see below), with a sterile no. 1 size cork borer. The sites for inoculation were marked on the bottom of each dish with the aid of a stencil, and the inoculation carried out with the ten-point inoculating needle already described (Garrett, 1946*b*). The plates were incubated for two months at 25° C., and the ten colonies were then cut out of each plate and buried in 2 lb. glass jam jars of soil. The soil employed in all the experiments was a mixture of one part (by volume) of Harpenden allotment soil with three parts of sand, and was maintained at a moisture content of 50 % saturation; depth of soil below the colonies was 1 in., and above, 2 in. Methods of preparing and handling soil were described in the preceding paper (Garrett, 1946*a*). After three to four months in the soil, the ten colonies were washed free of soil over a small 3 mm. sieve, and tested for viability by inoculation of carrot seedlings grown in the glasshouse.

For this purpose, stump-rooted carrot seedlings were grown in eleven equidistantly spaced groups of four plants each in wooden flats (14 × 8½ × 3 in.) of the same 1 soil : 3 sand mixture as used in the jars. This soil/sand mixture was known to be more favourable for growth of the mycelial strands of *H. purpureum* than undiluted soil (Garrett, 1946*a*), and a light-textured soil is generally considered to be specially favourable for growth of carrots. The planting of eleven groups of seedlings per box gave one group as a spare, and also permitted a more symmetrical arrangement of groups within the box. Seed of Early Market stump-rooted carrot was sown around the periphery of circular depressions 1 in. in diameter, and seedlings were thinned to four per group after three weeks. Sodium nitrate and potassium phosphate were applied in 100 ml. water per box, each at the rate of 2 cwt. per acre (1·86 g. per box) at three and seven weeks after sowing. Groups of seedlings were inoculated with the colonies of *H. purpureum* to be tested at the age of one month; a vertical slit wide enough to receive the colony was made in the soil in the centre of each group of seedlings, and closed up again after insertion of the colony. After inoculation, the seedlings were allowed to grow for another two months, and were then washed free of soil for examination of the tap roots; the number of colonies (out of ten) that had produced visible infection was recorded for each box. The lesions produced by *H. purpureum*, with their characteristic infection cushions, were unmistakable even to the naked eye; frequently, the whole of the tap root was infected.

EXPERIMENTAL

Experiment I. An attempt was made to determine the optimum period for incubation of colonies on the agar plates at 25° C. The three different agar media employed were variations of the meat-malt agar found by Buddin and Wakefield (1924) to be the best of any that they tested for growth of *H. purpureum.* The standard formula taken for this agar was as follows: Lemco beef extract, 10 g.; malt extract, 30 g.; agar, 20 g.; and distilled water, 1 l. In this experiment, the three agars chosen for comparison were malt agar (0 meat : 1 malt), normal strength meat-malt agar (1 meat : 1 malt), and double strength meat-malt agar (2 meat : 2 malt). Each agar was poured both 3 mm. deep (20 ml. per plate) and 6 mm. deep (40 ml. per plate), making six comparisons in all. After inoculation with the ten-point inoculating needle, plates were incubated at 25° C. for periods of three, six, nine and fourteen weeks; after each interval, two plates were taken from each of the six series for inoculation of the test carrot seedlings.

Percentages of viable colonies at each test are shown in Table 1. The results indicate that a decline in viability of colonies occurs when they are incubated at 25° C. for a period exceeding nine weeks. An incubation period of two months at 25° C. was therefore selected as a standard for further experiments. No great or consistent increase in viability was secured by pouring the agar at double depth, so that the standard depth of 3 mm. was selected for further experiments.

Experiment II. Colonies were grown on malt agar and on meat-malt agar for two months at 25° C., and were then buried in soil for periods of three and nine months before testing on carrot seedlings. Four jars, i.e. forty colonies, were taken as a sample from each series to be tested; results are shown in Table 2.

The difference in survival of colonies on malt and meat-malt agars is very pronounced, and is consistent with observations made when colonies were recovered from the soil, preparatory to inoculation of carrots. The colonies on meat-malt agar consisted of mycelial mats, with relatively small and undeveloped sclerotia. In the colonies on malt agar, the central inoculum disk usually carried one or more large sclerotia, or a sclerotial aggregate, firm and resilient in texture.

Table 1. *Percentage viable colonies*

Type and depth of agar	Age of colonies (weeks) 3	6	9	14
0 meat: 1 malt, shallow	50	90	90	40
0 meat: 1 malt, deep	75	90	100	70*
1 meat: 1 malt, shallow	85	85	75	5
1 meat: 1 malt, deep	95	100	100	10
2 meat: 2 malt, shallow	100	100	95	60
2 meat: 2 malt, deep	100	100	80	60

* 10 colonies only in test

Table 2. *Percentage viable colonies*

	Period of burial in soil (months) 3	9
Malt agar	75	68
Meat-malt agar	8	—

Table 3. *Percentage viable colonies*

Concentration of malt in agar	Ratio of meat: malt in agar 0:1	1:1	2:1
Half strength	18	8	0
Normal strength	93	5	13
Double strength	98	0	0

Experiment III. In this experiment, three different ratios of meat to malt constituent were tested, viz. 0 meat: 1 malt, 1 meat: 1 malt, and 2 meat: 1 malt (1 meat: 1 malt representing the standard ratio of 1 g. meat to 3 g. malt, and not equal parts by weight). Each ratio was made up at three different malt concentrations, viz. half normal, normal and twice normal strengths. After incubation of the inoculated plates at 25° C. for two months, colonies were buried in soil and left there for four months, after which period forty colonies from each series were tested by inoculation of carrot seedlings (Table 3).

Results again show that addition of meat extract to the agar greatly reduced percentage survival of colonies. Reduction of malt agar to half strength also markedly reduced percentage survival. These results are again consistent with the appearance of colonies at time of recovery from the soil. In those on malt agar, firm and resilient sclerotia, or sclerotial masses, had developed around the inoculum disk, this development being greatest on the double strength malt agar. Few or small sclerotia were observed on the meat-malt agars. Persistence of the mycelial felt was much better on the 1 meat:1 malt agars than on the 2 meat:1 malt agars. On the double strength 2 meat:1 malt agar (i.e. 4 meat:2 malt), the mycelial mats had disintegrated to a considerable extent in three out of the four soil jars.

A possible interpretation of these results is suggested by the observation that addition of meat extract (nitrogen content 8·85 %) to the agar increases the density of mycelial growth. This must occur at the expense of the carbohydrate reserves required for sclerotial production by the fungus colony. Moreover, when the colony is buried in the soil, the excess of nitrogen must encourage the growth of soil micro-organisms, which will then deplete still further the reserves of carbohydrate available for *H. purpureum* in the agar substrate.

Experiment IV. In this experiment, half strength malt agar (i.e. 1·5 % malt extract), with and without the addition of 1 % dextrose, was made up with 0, 0·125, 0·25 and 0·5 % sodium nitrate. Ten plates of each of the eight agars were inoculated in the usual way, and incubated for two months at 25° C. Colonies were then buried in soil for sixteen weeks, after which period ninety colonies from each of six series (two series were omitted from the test) were tested by inoculation of carrot seedlings (Table 4).

Table 4. *Percentage viable colonies*

	Percentage $NaNO_3$ in agar			
	0	0·125	0·25	0·5
1·5 % malt + 1 % dextrose	42	4	0	13
1·5 % malt alone	11	4	—	—

These results show that both omission of dextrose and addition of sodium nitrate have depressed survival of *H. purpureum*, and therefore support the interpretation placed upon the results of Exp. III. Once again the results of this experiment could have been forecast, very approximately, from the appearance of the colonies at time of recovery from the soil. Colonies on 1·5 % malt + 1 % dextrose showed the best development of firm sclerotia, those on 1·5 % malt without dextrose being much poorer. Few or no sclerotia were formed on agars to which sodium nitrate had been added. The maximum survival of colonies in this experiment was only 42 %, on 1·5 % malt + 1 % dextrose; this figure is intermediate between that on 1·5 % malt (18) and that on 3 % malt (93) in Exp. III.

Experiment V. This experiment was designed to investigate the effect of a wider range of carbohydrate and nitrate nitrogen concentrations than that employed in Exp. IV. The basal medium employed was as follows: malt extract, 15 g.; potassium phosphate, 1 g.; magnesium sulphate (crystalline), 0·5 g.; agar, 20 g.; and distilled water, 1 l. Malt extract was assumed to supply all possibly essential growth factors (the growth-factor requirements of *H. purpureum* have not been determined). The mineral nutrients were added to ensure that growth of the fungus was not limited by nutrients other than carbohydrate and nitrogen. To samples of the basal medium were added, respectively, 1, 2 and 4 % dextrose. Each of the three dextrose series was made up into six subordinate series, with the following percentages of nitrogen as sodium nitrate: 0, 0·01, 0·02, 0·04, 0·08 and 0·16 (with the single exception that the series 1 % dextrose + 0·16 % nitrogen was omitted, as likely to lie well beyond the range of interest). Three plates were poured with each of the seventeen agars, inoculated, and incubated for eight weeks at 25° C. The thirty colonies of each series were next buried in soil for a period of two months, and then tested for viability by inoculation of carrot seedlings. Unfortunately it was impossible to allow colonies to remain in the soil for the longer period of three to four months employed in the previous experiments. The experiment was not started until 29 May, and the carrots were inoculated on 22–24 September; had inoculation been delayed much longer, the carrots would not have made sufficiently good growth under the deteriorating light conditions. Results are given in Table 5.

Table 5. *Percentage viable colonies*

Percentage nitrogen	Percentage supplementary dextrose		
	4	2	1
0	37	37	16
0·01	73	30	50
0·02	50	40	23
0·04	47	53	17
0·08	73	13	3
0·16	70	30	—

Inspection of these results shows that the optimum level of nitrogen for survival of colonies increases with the concentration of dextrose: it is 0·01 % nitrogen for 1 % dextrose, 0·04 % nitrogen for 2 % dextrose, and extends up to 0·16 % nitrogen (the highest level included in the experiment) for 4 % dextrose. These figures support the view, advanced above, that the depressing effect of nitrogen upon colony survival is due to more rapid consumption of available carbohydrate by the denser mycelial growth, induced by a higher nitrogen content of the medium. These figures are also quite consistent with those given in Tables 2 and 3, showing that percentage colony survival was low on meat-malt agar, both at normal and at double strengths. An approximate calculation shows that, to secure a ratio of nitrogen to carbohydrate equivalent to that in meat-malt agar, it would be necessary to supply 0·10 % nitrogen to the 1 % dextrose series, 0·15 % nitrogen to the 2 % dextrose series, and 0·25 % nitrogen to the 4 % dextrose series (the malt extract contains 73 % dry matter, and 0·85 % nitrogen on a wet basis: the meat extract contains 8·85 % nitrogen on a wet basis).

Caution is obviously necessary in drawing conclusions from an unfinished investigation on the survival of *H. purpureum* colonies on an agar substratum. The observed effects of increased carbohydrate concentration (both in the form of malt extract and of dextrose) in increasing survival, and of nitrogen (both as meat extract and as sodium nitrate) in reducing it, may be tentatively ascribed to their visible effects on the formation of sclerotia. This phenomenon, however, is quite possibly a laboratory artefact, without parallel in the field. On the culture plate, the carbohydrate reserves, whether in the form of malt extract or of dextrose, are readily available, and may therefore be rapidly consumed in the early stages of colony growth, provided that adequate supplies of nitrogen and other essential nutrients are present. On the host plant, on the other hand, the food reserves open to the fungus must usually be adequate for formation of viable sclerotia, for the very reason that the developing sclerotia can probably continue to draw on those food reserves that are not too readily available, and therefore cannot be exhausted during the earlier phases of mycelial growth.

A possible parallel to these results is afforded by the observations of Geach (1936) working with cultures of *Aphanomyces euteiches*, the causal agent of pea root rot. Geach found that on 2 % cornmeal agar, mycelial growth was sparse, but production of oospores was abundant; on 2 % cornmeal + 2 % peptone agar, on the other hand, mycelial growth was dense, but no oospores were produced. In the absence of oospores, cultures lost their viability rather rapidly, and no growth was made by transfers from cultures thirty-three days old. Oospore formation was also inhibited by the addition to 2 % cornmeal agar of 0·4 % ammonium sulphate or of 0·5 % sodium nitrate.

SUMMARY

Plates of different nutrient agars were inoculated at ten equidistant points with *Helicobasidium purpureum* and incubated for two months at 25° C.; the fungus colonies were buried in jars of soil for three to four months, and then tested for viability by inoculation of carrot seedlings. Survival was increased by raising the carbohydrate concentration of the medium, but depressed by excess of nitrogen. The optimum nitrogen requirement for survival increased with rise in carbohydrate content of the medium. Survival of colonies was correlated with the production of firm resilient sclerotia around the centre of the colony. The depressing effect of excess nitrogen upon production of sclerotia and survival of colonies is attributed to an increased density of mycelial growth, leading to reduction of carbohydrate level below that required for maturation of viable sclerotia.

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THE MORPHOLOGY OF *PENICILLIUM CHRYSOGENUM* IN SUBMERGED FERMENTATIONS

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(With Plates XVIII and XIX and 10 Text-figures)

INTRODUCTION

Several papers have been written during the last few years on the morphology of the moulds *Penicillium notatum* and *P. chrysogenum* used for the production of penicillin. Of these the paper by Raper and Alexander (1945) describing the most important of the strains used is of particular interest. These workers, however, dealt almost exclusively with surface cultures of the moulds; the morphology of these organisms in submerged culture still awaits attention. The effects of agitation on the morphology of fungi were first investigated over fifty years ago, when Russell (1892) observed that such conditions favoured a bud-like rather than the more normal filamentous form. A few years later, Ray (1897) devoted a chapter in his thesis to the influence of agitation on *Sterigmatocystis alba* (*Aspergillus candidus* group). Of particular interest were his conclusions that: (*a*) the rate of growth in agitated culture is higher than in similar still cultures; (*b*) spherical colonies having a pseudoparenchymatous structure are formed; (*c*) atypical reproductive structures and a 'forme de conservation résistante' occur.

In 1908 Sartory produced a comprehensive thesis on the effect of agitation on species of the Mucorales, Ascomycetes and Fungi Imperfecti. He included three species of *Penicillium.* His investigation was more detailed than those previously undertaken and included sectioning of the colonies and the isolation and growth of various anomalous structures. While agreeing in the main with Ray he introduced certain new generalizations, namely, that (*a*) increase in the rate of agitation accentuates malformation of the hyphae and, in particular, budding; (*b*) the spherical form of colony is not universal; (*c*) colonies fragment; (*d*) abnormal forms, when grown in surface culture, pass through a series of stages back to the normal.

Kluyver and Perquin (1933) published a paper on *Aspergillus flavus* in which they emphasized the high metabolic rate obtained in agitated and aerated conditions. Little further work on the structure of submerged cultures was described until Burkholder and Sinnott (1945) published a general account of the morphology of a wide range of fungi which they grew in shaken culture. Among these were *Penicillium notatum* and *P. chrysogenum.* As was general under such conditions, globose colonies were obtained.

The importance of the type of culture produced is generally recognized by manufacturers of penicillin. Moyer and Coghill (1946) comment on the fact that certain types of growth are more favourable than others for the production of high yields of penicillin. This fact soon became apparent in the course of experimental work in our laboratories, and accordingly the morphology of *P. chrysogenum* under conditions of submerged fermentation has been made the subject of intensive investigation. In this communication the morphological changes which take place in typical cultures grown under standardized conditions are described and discussed.

EXPERIMENTAL METHODS

Commercially, it has generally been found advantageous to carry out the penicillin fermentation in two stages. In the first stage, conidia of the mould are grown in a medium containing glucose and corn-steep liquor to produce a mycelial inoculum. In the second stage, this mycelial inoculum is transferred to a medium containing lactose and corn-steep liquor (the penicillin fermentation).

The organism. The culture used was derived from a strain of *P. chrysogenum*, Q 176, which was prepared at the University of Wisconsin, Madison, Wisconsin.

Media. The media used were as follows (all percentages are w/v):

(*a*) Mycelial inoculum:	%
Glucose chips	6
Corn-steep-liquor solids	2
Chalk	0·5
Water to	100

(*b*) Penicillin fermentation:	%
Lactose B.P.	2
Corn-steep-liquor solids	2
Chalk	0·5
Phenylacetic acid	0·15*
Water to	100

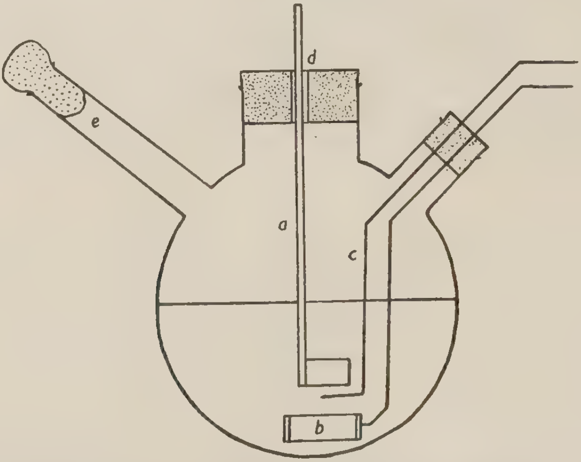
* Added in three lots at 0, 24 and 48 hr., dissolved in the theoretical quantity of sodium hydroxide solution.

The Mycelial inoculum. 750 ml. conical flasks containing 100 ml. of medium were inoculated with 10^8 conidia of the mould. The conidia were grown on a glycerol-molasses-peptone-agar medium (cf. Frank, Calam & Gregory, 1948), suspended in water, and their concentration estimated by means of a haemocytometer. The inoculated flasks were incubated at 23–25° C. on a shaking machine which imparted to each flask a rotary motion. Each flask completed 100 cycles of 3 in. diameter per minute.

The Penicillin fermentation. Two-litre 'Pyrex' bolthead flasks fitted with a single-bladed paddle agitator ($\frac{3}{4}$ in. high by $1\frac{1}{2}$ in. long) rotating at 600–650 r.p.m. were used (Text-fig. 1). The stirrer-shaft entered the neck of the flask by means of a gland designed to exclude micro-organisms. Additional provision was made for the admission of an air-pipe, for inoculation and for sampling. Air was passed in at the rate of 1·2 l./min., and was dispersed by a length of porous tubing (Aerox Ltd., P 28, 1 in. long, $\frac{7}{8}$ in. diameter, $\frac{1}{2}$ in. bore). The flasks each contained 1·1 l. of medium. Excessive foaming was prevented by the addition, from time to time, of arachis oil containing 3 % octadecanol.

Penicillin fermentations were inoculated by the addition of a forty-eight-hour mycelial inoculum (100 ml.) prepared as described above.

Sampling and preparation of specimens. Five-millilitre samples were taken from the cultures at frequent intervals and fixed in 10 % formalin. Slide preparations were stained with cotton blue in lactophenol.



Text-fig. 1. Bolthead culture vessel used for laboratory scale penicillin fermentations. *a*, flag stirrer. *b*, porous tube to distribute incoming air. *c*, tube for sampling. *d*, bearing designed to exclude micro-organisms. *e*, tube for inoculation and outlet for air.

HYPHAL DEVELOPMENT IN THE MYCELIAL INOCULUM MEDIUM

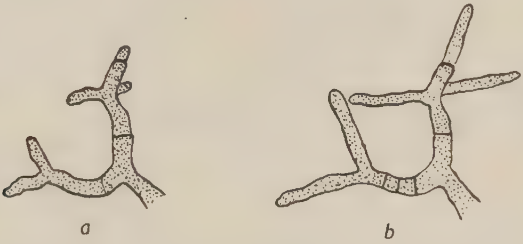
Germination of conidia

When suspended in water the conidia of *Penicillium chrysogenum*, Q 176, vary in diameter from 3·2 to 8·3 μ, averaging 4·6 μ. Thom (1930) found occasional abnormally large conidia in many species of *Penicillium*. These were so rare that he ignored them when giving the range of conidial size. In our suspensions these large conidia occur to the extent of about 0·5 %. In agitated medium the conidia begin to swell. This swelling ceases temporarily at the time of germination, at which stage most of them are between 5 and 7 μ in diameter, though the original abnormally large conidia are naturally greater in diameter. Swelling of this order for conidia germinating on agar media has been mentioned by Thom (1930) in *Penicillium* spp. generally and by Dr P. W. Brian (private communication) in *P. expansum*. Dr Brian also states that this swelling ceases when percentage germination has reached a high level.

In our conditions, 50 % germination occurs consistently between the seventh and eighth hour (Davies, Duckworth & Harris, 1948) and by the twelfth hour about 95 % of the conidia have germinated. Grenfell, Legge & White (1947) estimated that only about 45 % of the conidia, which they were using to inoculate penicillin fermentations, were viable. The discrepancy is probably accounted for by the different methods of assessment employed, since the plating method used by these workers would probably give low results unless multiconidial colonies, loss of conidia during transfer and desiccation of spores were avoided.

Germination of conidia of the *Penicillium notatum-chrysogenum* group within agitated media appears to be more rapid than on the surface of solid or liquid media (Baldwin & Harris, unpublished).

The conidia in the original suspension are separate but they soon become attached in groups. The effect of grouping on the organization of the mycelium, for example into spherical colonies, will be discussed in a later communication. Most of the conidia are spherical and germinate by the formation of a protuberance which rapidly increases in length to form a germ-tube 4–5 μ wide. This tube stains more rapidly than either the parent conidium or other ungerminated conidia. At the time of germination almost all the conidia produce one germ-tube only, but occasionally two or very rarely three or four tubes are produced simultaneously. It is usual for multiple germ-tubes to grow out equidistant from each other in the same plane. Cytological changes occurring at the time of germination are being investigated.



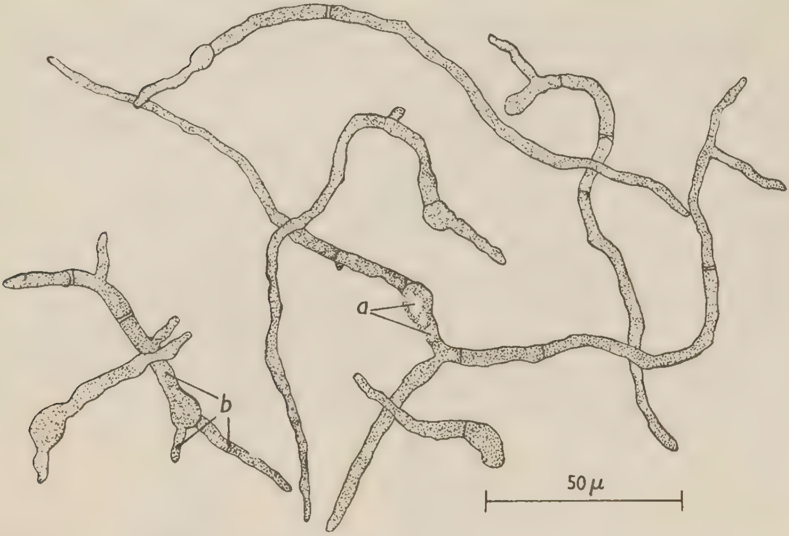
Text-fig. 2. Region of growth. *a*, part of a growing colony of *Penicillium chrysogenum*, Q176. Notice position of septa. *b*, the same colony three hours later, showing growth of the apical regions only.

Normal hyphae. Growth is confined to the terminal 'cells' of the original germ-tubes and of side branches (Text-fig. 2). Two or three per cent of the germ-tubes have produced a branch by the twelfth hour, and by the fifteenth hour branches are common (Text-fig. 3 and Pl. XVIII, fig. 1). Later they are produced at irregular intervals along the parent hypha. Each branch normally arises from a region just behind a septum and is set at an angle of 70–90° to the direction of growth of the parent hypha.

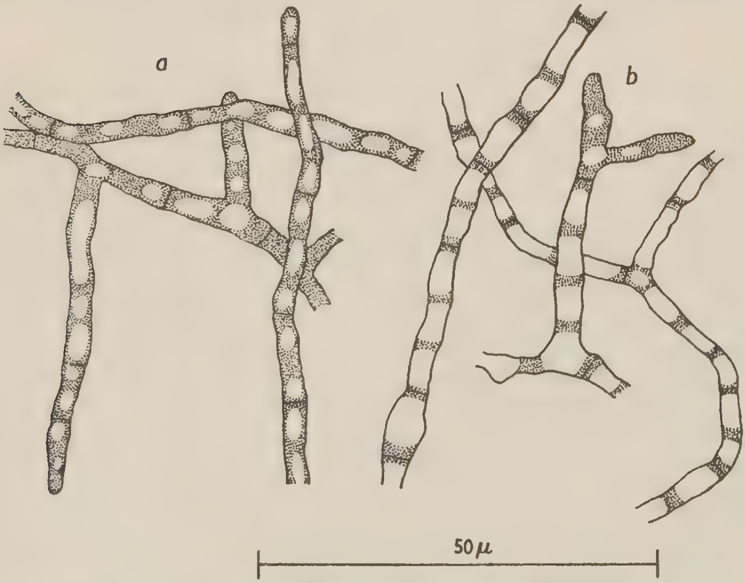
Most of the hyphae in the young culture elongate at a rate of about 30 μ per hour, though, when germ-tubes are formed serially from a single conidium, the later ones frequently do not finally exceed 15 μ in length.

Very small vacuoles can be seen in some germ-tubes at twelve hours. These vacuoles, which later increase in size and frequency, are usually oval in outline and appear as clear, sharply defined areas in the densely staining cytoplasm (Text-fig. 4*a*, 'chain vacuolation'). A rapid increase in vacuolation occurs between the twenty-fourth and thirtieth hours, and during this period short regions between septa become completely devoid of cytoplasm.

It seems probable that the formation of chains of oval vacuoles is a stage in the normal maturation process of this organism. A further stage in the process, known but seldom seen in the inoculum culture, is shown by an



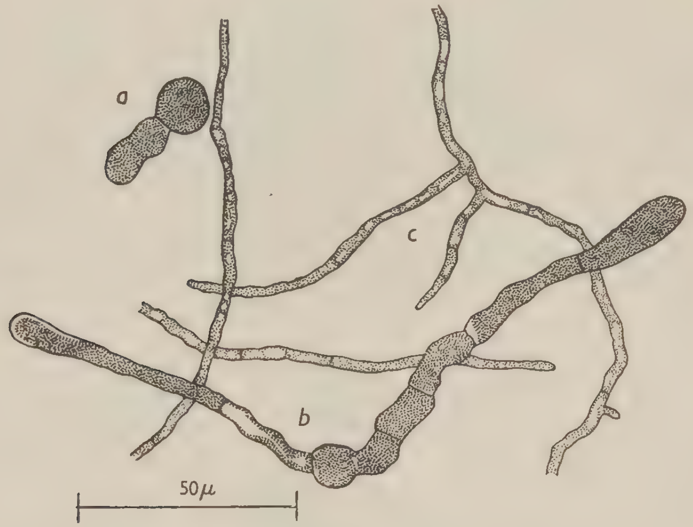
Text-fig. 3. Hyphal appearance at the fifteenth hour in the inoculum medium. *a*, vacuoles. *b*, three germ-tubes arising from one spore.



Text-fig. 4. *a*, 'chain vacuolation'. Rows of round or oval vacuoles giving a characteristic appearance. *b*, 'ladder vacuolation'. The vacuoles are larger and fill entire regions of the hyphae.

increase in the size of the vacuoles so that they almost completely fill the hyphae (Text-fig. 4*b* 'ladder vacuolation'). More commonly the unvacuolated or 'chain-vacuolated' contents withdraw from the walls and finally disappear.

The width of the young hyphal regions just behind the tip decreases during the course of the fermentation. This is noticeable at the eighteenth hour (*c*. 3·5 μ), is very obvious between the twenty-first and forty-eighth hour in the mycelial inoculum medium (*c*. 2·5 μ) and, as will be seen later, is still more pronounced in the penicillin fermentation (*c*. 1·5 μ). Branches produced from the younger regions of the hyphae are themselves narrower, less frequent and usually set at a smaller angle to the direction of growth of the parent hypha, than are those formed earlier.



Text-fig. 5. Late-germinating spores, producing wide densely staining outgrowths at the thirtieth hour in the inoculum medium. *a*, newly germinated swollen spore. *b*, one which has produced two germ tubes. Notice the withdrawal of the hyphal contents and the typical clavate tips. *c*, normal hyphae showing considerable vacuolation.

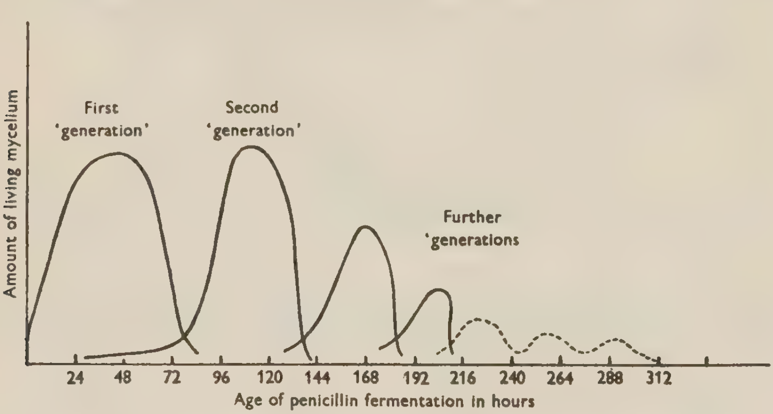
Other hyphal forms. In addition to the normal hyphae, certain hitherto undescribed structures are formed in the later stages of the mycelial inoculum culture. These originate from the 5 % of conidia which do not germinate during the usual period (four to twelve hours), but which continue to swell until by about the twenty-seventh hour they are 10–12 μ in diameter. They are more frequent than, and therefore not identical with, the very large conidia in the original suspension. At about this time they germinate and produce one or two unbranched outgrowths, 7–8 μ wide, with very densely staining contents and occasional septa. The ends of these outgrowths are frequently swollen and clavate in outline and are about 10 μ wide. These forms contrast strikingly with the normal hyphae at this stage (Text-fig. 5 and Pl. XVIII, fig. 2). Withdrawal of the cytoplasm from the walls is more marked in these wide forms than in the normal hyphae. The little growth which occurs appears to be intercalary, not terminal, and is accompanied by a slight reduction in width.

During the later stages of the mycelial inoculum culture otherwise normal hyphae occasionally bear stunted swollen ends. These cannot be confused with the wide forms in which the contents are more homogeneous and stain more deeply.

HYPHAL DEVELOPMENT IN THE PENICILLIN FERMENTATION

Since the following observations are novel and relatively complex, a brief outline of the macroscopic changes occurring in the penicillin fermentation will first of all be given.

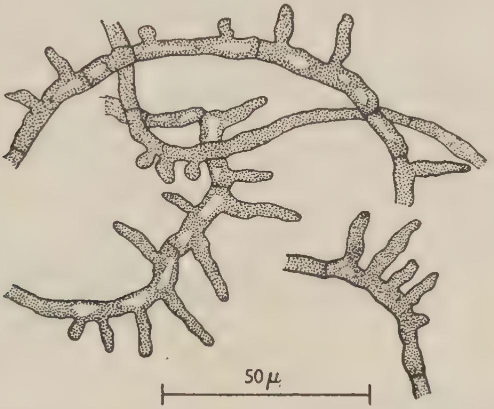
The mycelial inoculum grows vigorously until the entire volume of medium becomes dense with hyphae. The whole culture is then thick and moderately resistant to agitation. For convenience this may be described as the first 'generation' of hyphae. Within a few hours death of the greater portion of this 'generation' of hyphae results in the culture again becoming thin and mobile. A second 'generation' of mycelium arises from persistent elements of the first. Again a thick 'brei' is formed—the connotation is mash, porridge or pulp. Similar fluctuations, less pronounced in character, occur before the culture finally degenerates (Text-fig. 6).



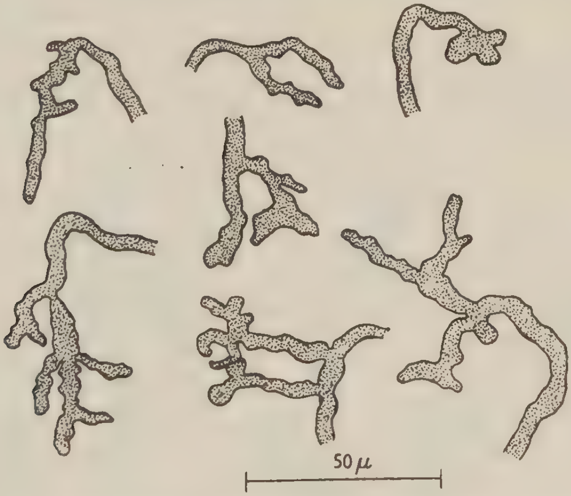
Text-fig. 6. The phases of growth occurring in the penicillin fermentation.

First 'generation'. When the mycelial inoculum is added to the penicillin-fermentation medium, numerous side branches rapidly develop on the existing hyphae (Text-fig. 7). These usually occur in groups very close together and may be 30 μ long after three hours in the new medium (Pl. XVIII, fig. 3). After nine hours' stirring and agitation these side branches, some of which themselves possess branches, reach a length of 250 μ. Their rate of growth is a little higher than that of newly germinated conidia in the mycelial inoculum culture.

Rapid growth of the normal hyphae continues for about sixty hours, during which time the hyphal tips become narrower and decrease gradually to a width of less than 2 μ. The medium becomes filled with hyphae which produce a thick suspension or 'brei' (Pl. XIX, fig. 6). Before this occurs many of the hyphal tips show, for a short time, a peculiar distorted appearance (Text-fig. 8). This effect is similar to that reported by Brian, Curtis and Hemming (1946) as being produced by the action of 'curling factor' on *Botrytis allii*, but in our cultures this appearance is transitory.



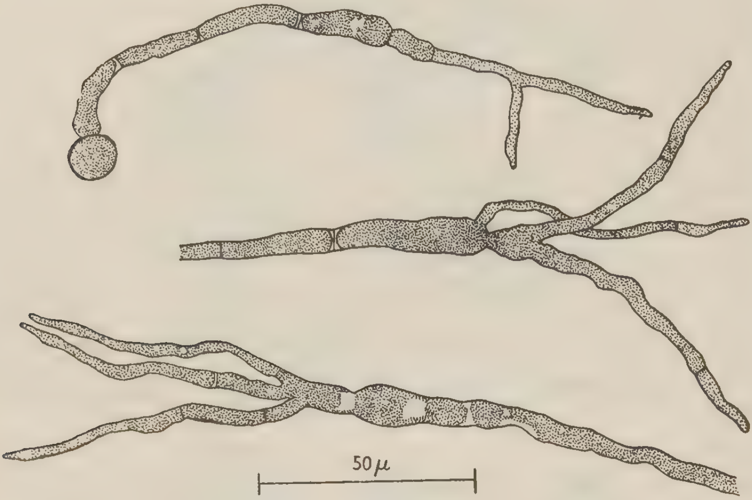
Text-fig. 7. The formation of numerous, grouped side-branches after three hours in the penicillin medium.



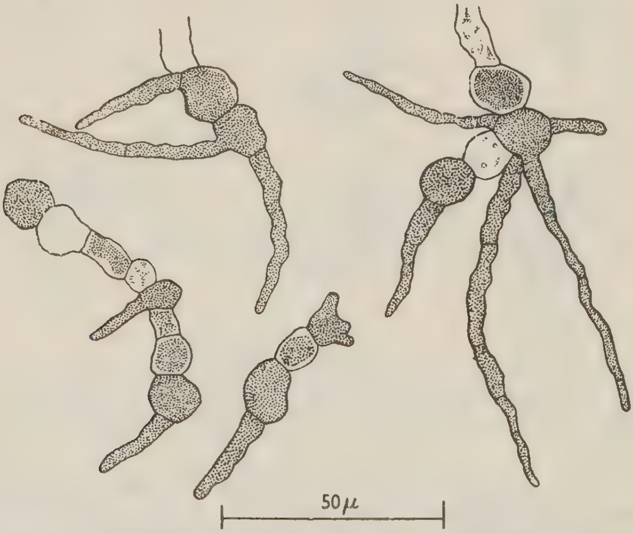
Text-fig. 8. Distorted stunted hyphal tips found at about the eighteenth hour in the penicillin fermentation.

During this period empty hyphal walls are comparatively rare, but they rapidly become very common between the seventy-second and the seventy-eighth hour after inoculation. Since the walls themselves are not rigid the whole culture becomes thin and mobile.

The wide forms present in the mycelial inoculum develop in the penicillin-fermentation medium in some or all of the following ways: (*a*) further intercalary growth occurs so that at nine hours they may have reached 300 μ in length; (*b*) one or two densely staining non-vacuolated outgrowths may be formed from the clavate end (Text-fig. 9): these are originally 5–8 μ wide but later become indistinguishable from normal hyphae; (*c*) branches from the intercalary regions develop and produce hyphae which are normal in appearance; (*d*) large spherical cells are formed along the wide hyphae, singly or in short chains and usually in an intercalary



Text-fig. 9. Outgrowths from clavate tips in the early stages of the penicillin fermentation.



Text-fig. 10. Groups of large spherical cells producing densely staining outgrowths.

position. Their appearance is similar to that of the swollen conidia from which the wide hyphal forms originally arose. Some of these spherical cells become devoid of contents, but many retain their densely staining cytoplasm and form one or more outgrowths (Text-fig. 10 and Pl. XIX, fig. 5).

Second 'generation'. It is from these large spherical cells that the second 'generation' is largely produced (Pl. XIX, fig. 7). The outgrowths from them are at first 4–5 μ wide with densely staining contents and characteristically wavy walls, but as the first 'generation' dies off, they grow rapidly, branch and vacuolate. By ninety-six hours the macroscopic and microscopic appearance of the culture, apart from the presence of many empty walls, is very similar to that of the same culture at the fortieth hour (Pl. XIX, fig. 8). The hyphae of the second 'generation' become progressively narrower and more vacuolated so that by the hundred and eighth hour some, and by the hundred and thirty-second hour practically all, are empty. The culture then seems dead. However, even at this stage, another generation grows up and further, though less pronounced, fluctuations in the amount of living material follow, until, after the two-hundredth hour, the culture finally degenerates.

DISCUSSION

This communication is mainly descriptive and little attempt has been made to explain the phenomena observed; it is intended as an introduction to work on the isolation of specific factors responsible for the formation of the above-described characteristic structures. Some particular points are considered below.

A striking feature in these cultures is the diversity of mycelial form. The wide hyphae with clavate tips, the distorted endings and the large spherical cells are structures never previously associated with this species. In that they materially expand our knowledge of the morphology of the organism, the new forms here described raise a taxonomic problem, but it is perhaps better to suspend judgement on their significance.

It is possible that conidia which, by chance, germinate at a later stage in the inoculum media form abnormal hyphae as a result of some environmental condition produced by the growing mycelium. It is equally possible that these conidia are of a spontaneous variant which later reverts to the more vigorous parental type (Sartory, 1908; Sansome, 1947). Further work on this point is necessary.

The distorted endings occurring in the early stages of the penicillin fermentation are thought to be due to the vigorous nature of the agitation-aeration system. As the culture becomes more dense they rapidly disappear. These endings are similar in appearance to the 'degenerate reproductive structures' described and illustrated by Sartory in other species of *Penicillium* grown under conditions of agitation. However, penicilli, the elements of which are typical in size and dimensions though not in organization, do occur in the same cultures as distorted endings similar to those which we have described. It therefore appears probable that these distorted endings are the effect of environmental factors on vegetative hyphae and are not, as Sartory suggested, atypical penicilli.

The spherical cells differ from the 'forme de conservation résistante' observed by Sartory in that they possess no oil reserves and have walls which are only slightly thicker than normal. On morphological grounds they cannot be regarded as chlamydospores. On the other hand, in that they tide the organism over periods during which conditions are unfavourable, they play the same physiological role.

The successive phases which occur in the penicillin fermentation show that it is not, as might perhaps be expected, a simple process involving the growth, maturation and senescence of the culture as a whole. The early rapid growth is followed by death of most of the hyphae, but persistent elements, the spherical cells which are directly descended from characteristic forms in the inoculum culture, form a new 'generation'. An explanation of these facts along the following lines was entertained.

As the amount of mycelium in the medium increases, the amount of oxygen available per living 'cell' falls, until a value is reached at which the oxygen requirements of the culture as a whole cannot be met. Presumably as a result of this, many of the 'cells' die, and in consequence a sudden large increase in the number of empty hyphal walls follows. This causes the culture to become thin and mobile. The oxygen supply to the residual living population is then adequate for renewed growth. The later cycles may be explained in the same way, though, in the impoverished medium, they will be less pronounced. In point of fact, data relating to oxygen transfer between air, medium and mycelium, subsequently obtained, support this explanation.

This description forms a basis upon which we hope to enlarge in future communications. It is hoped to examine the effect of environmental conditions on the morphology of agitated cultures of the *Penicillium notatum-chrysogenum* group. With such information available, it may be possible to define, by morphological examination, the conditions under which cultures have been grown and to correlate microscopic appearance with biochemical behaviour.

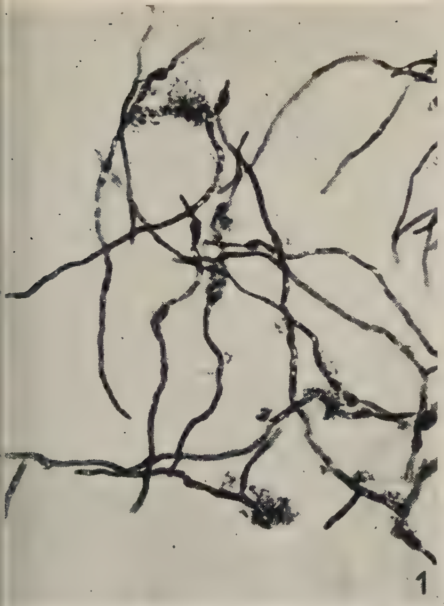
SUMMARY

Conidia of *Penicillium chrysogenum*, Q 176 were inoculated into a liquid nutrient medium contained in a conical flask, which was then mechanically shaken for forty-eight hours (the mycelial inoculum culture). At this stage the mycelium so produced was transferred to a second liquid medium which was stirred and aerated in a bolthead flask (the penicillin fermentation). The morphology of these two cultures is described.

In the mycelial inoculum the majority of the conidia germinate between the fourth and twelfth hours to produce hyphae which branch and become progressively narrower. But some conidia (about 5 %) germinate later and form abnormally wide hyphae, usually with swollen tips.

The hyphae used to inoculate the bolthead flask rapidly produce, along their length, numerous side branches, growth of which soon fills the medium. Later these rapidly die. Meanwhile, the wide hyphae from the inoculum culture have formed spherical bodies which, at this stage, proliferate and form a second 'generation' of mycelium. Further fluctuations in the amount of living material follow until the culture finally degenerates.

The scope and aims of future work are indicated.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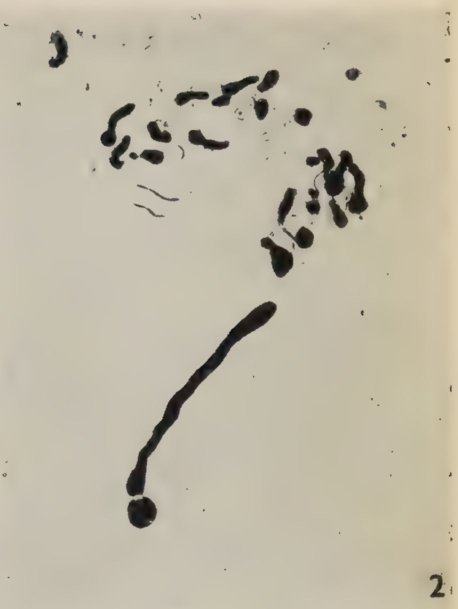
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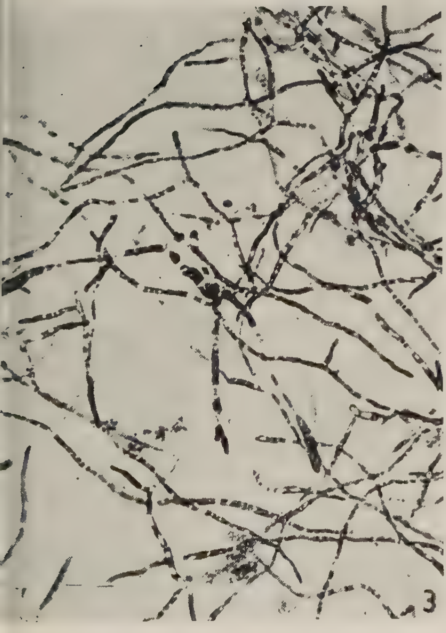
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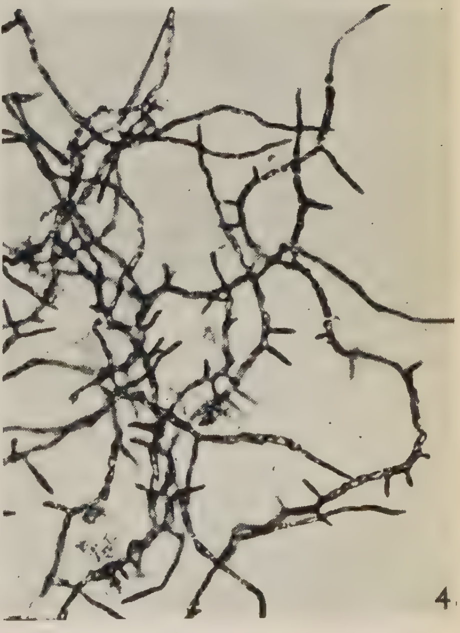
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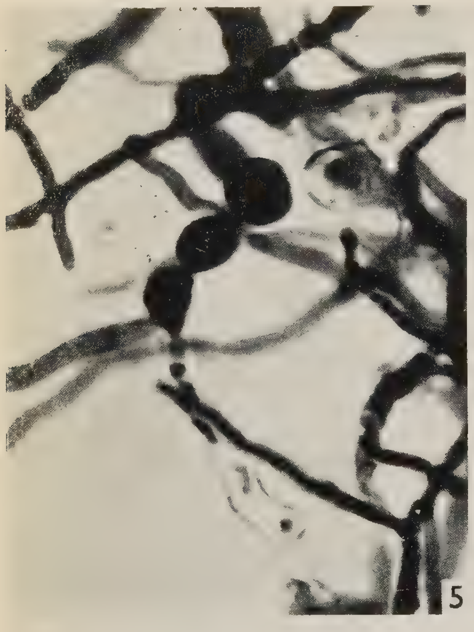
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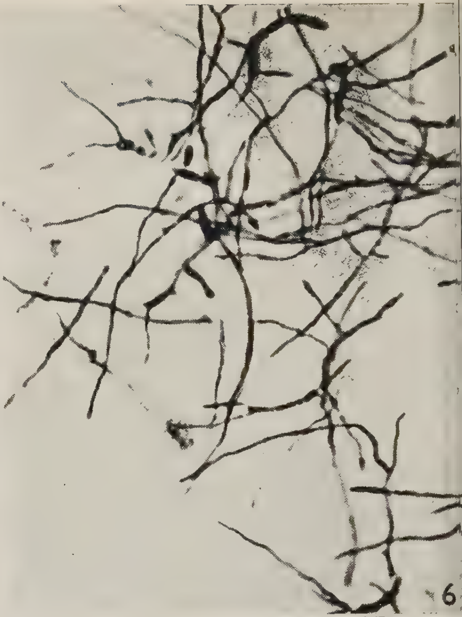
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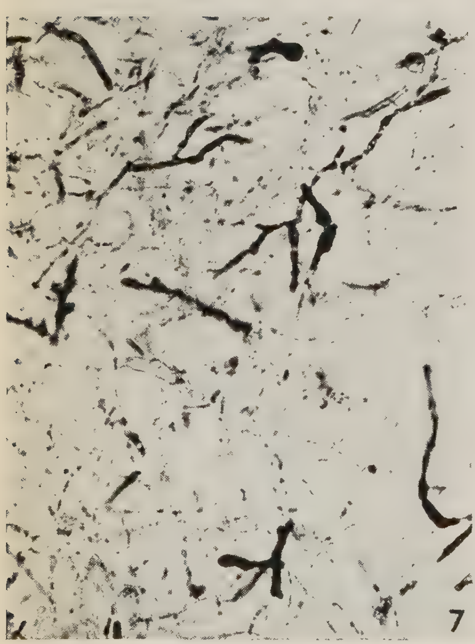
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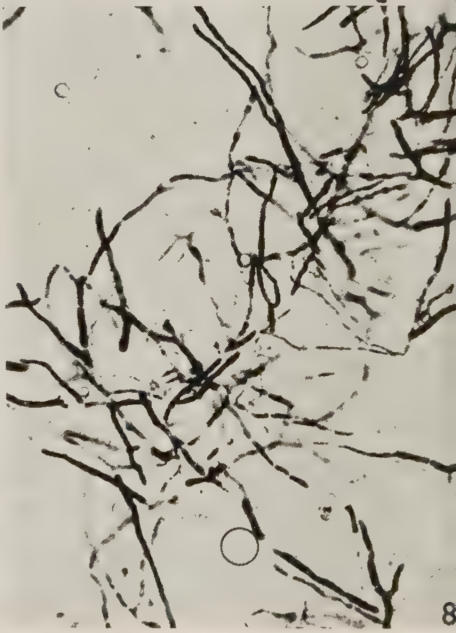
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EXPLANATION OF PLATES

Plate XVIII

Fig. 1. Hyphae from the inoculum medium at the fifteenth hour. × 300.

Fig. 2. A late-germinating swollen conidium at the thirtieth hour compared with conidia germinating during the normal period (four to twelve hours). × 600.

Fig. 3. Hyphae from the inoculum medium at the forty-eighth hour; a swollen spore with two outgrowths can be seen. Each outgrowth has a clavate densely staining tip. × 300.

Fig. 4. Hyphae from the penicillin fermentation after three hours; many short side-branches are present. × 300.

Plate XIX

Fig. 5. Large spherical cells from the penicillin fermentation at the forty-eighth hour. × 1100.

Fig. 6. Hyphae from the penicillin fermentation at the thirty-sixth hour. × 300.

Fig. 7. Hyphae from the penicillin fermentation at the seventy-eighth hour; many empty walls are present. × 300.

Fig. 8. Hyphae from the penicillin fermentation at the ninety-sixth hour; much new growth has taken place. × 300.

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STUDIES ON BRITISH CHYTRIDS

VII. ON *PHLYCTOCHYTRIUM MUCRONATUM* N.SP.

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(With 2 Text-figures)

This chytrid was found growing as a saprophyte on *Closterium pritchardianum* Arch., inhabiting the mud surface, in the Clay Pond, Wray Castle, from late September to the end of November 1946 and in January 1947 on *C. costatum* Corda. in Blelham Bog, Wray Castle, Lancashire. At present it is included in the genus *Phlyctochytrium* as a new species, *P. mucronatum*, but when the nature of the resting spore, if any, is known this position may have to be revised.

The following description is based on observations of a large number of living specimens. All attempts to culture the chytrid on dead *Closterium*, on other algae, on pollen grains, and on *Daphnia*, failed. The zoospore settles on the host cell and puts forth a germ tube which penetrates the wall (Fig. 1*e*) and soon begins to branch. The rhizoidal system elongates, branches further and the portion immediately within the algal wall enlarges to form a small (1·3–3·5 μ) spherical or subspherical apophysis. It seems that the apophysis is formed secondarily as a swelling of the germ tube after initiation of the rhizoids. Zoospores which had germinated in water under a cover-slip in the early stages of development showed no signs of an apophysis (Fig. 1*d*); however, they soon died and the subsequent formation of an apophysis could not be demonstrated. Two or three main rhizoidal axes may extend from the apophysis in large specimens, whereas small thalli may possess only one main branch. The extensive main axes often branch dichotomously, and taper towards their extremities.

The encysted zoospore is at first spherical with a single oil globule (Fig. 1*e*), it soon becomes more broadly ellipsoidal and often contains two oil globules (Fig. 1*f*). At first the apical spine is blunt (Fig. 1*g*), but as it elongates it becomes sharply pointed.

The subsequent changes in the protoplasm are similar to those found in most chytrids. It becomes finely granular, later small globules appear which coalesce to form the conspicuous oil globules of the zoospores. The mature sporangium exhibits a great variation in size and degree of ornamentation. An apical spine is always formed, and only once has a bifurcated specimen been observed (Fig. 1*n*). On the small sporangia 8–13 × 4–7 μ (Fig. 1*i*) no further spines develop, but as the sporangia become larger so the ornamentation increases. Some sporangia have, in addition to the apical spine, two oppositely placed median spines (Fig. 1*j*, *m*), others have from one to four whorls of spines. The very large specimens (31 μ in

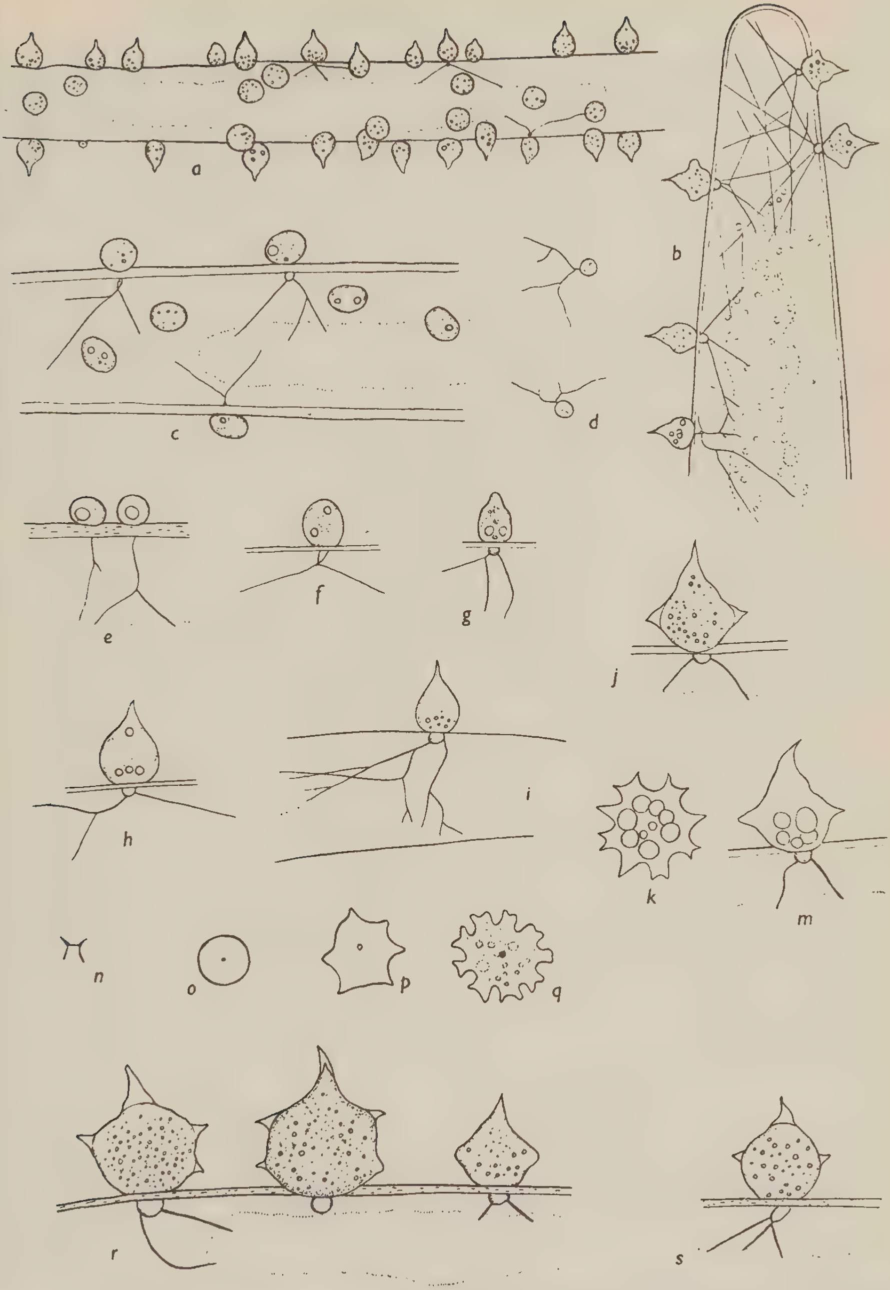

Fig. 1. *Phlyctochytrium mucronatum* n.sp. *a*, part of a *Closterium* cell with many young thalli. *b*, five larger sporangia, each with an apophysis and main rhizoidal axes. *c*, very young sporangia. *d*, early stage in germination of two zoospores under a cover-slip. *e–i*, stages in development of thalli with only an apical spine. *j*, immature thallus with two lateral spines. *k*, *m*, thallus with single median spine whorl; some spines in the whorl forked, others simple. *n*, forked apical spine. *o–q*, sporangia viewed from above. *o*, no median spines; *p*, simple spines; *q*, forked spines. *r*, *s*, larger thalli with one and two spine whorls. *a*, *b*, *d*, ×450; *h*, ×720; *c*, *e–g*, *i–s*, ×945.

diameter including the spines) with four spine whorls (Fig. 2*c*) are rare and usually the basal whorl is less well developed than the rest. The spines may be bifurcated or simple and both types may occur in the same whorl (Fig. 1*k*); only their extreme apices are solid. The size of the sporangium shows no correlation with the number of parasites occurring on the host cell. Small sporangia often occur in large numbers (up to 100) on a host cell, but there is no indication that they are impoverished specimens due to overcrowding. Large sporangia with two to four lateral whorls of spines develop beside them and isolated, small sporangia are common. When mature, the sporangium contains a number of spherical refractive globules each of which indicates the position of a zoospore. The number of zoospores formed in a sporangium varies according to its size; three to eight in small

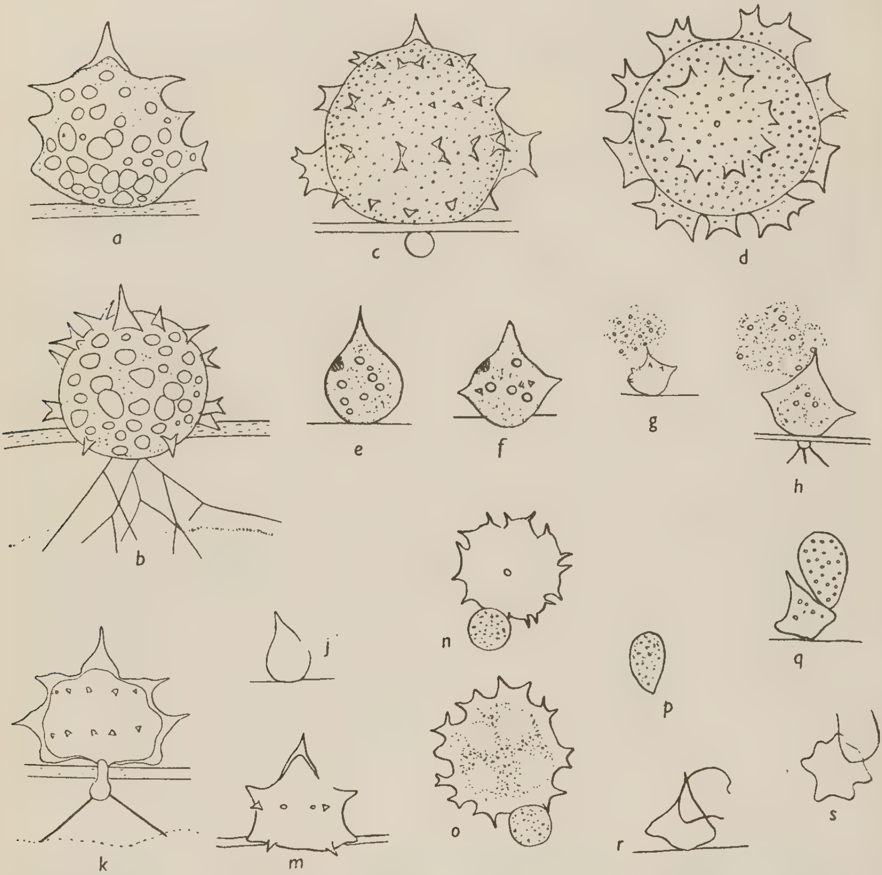

Fig. 2. *Phlyctochytrium mucronatum* n.sp. *a*, sporangium with three spine whorls. *b*, the same as (*a*) showing main rhizoidal axes. *c*, four-spine whorled thallus. *d*, (*c*) viewed from above. *e*, *f*, mature thalli with lateral dully refractive area marking the place of dehiscence. *g*, *h*, sporangia dehiscing. *j*, *k*, *m*, empty sporangia. *n*–*p*, hyperparasite in different views on the same sporangium. *q*, mature sporangium. *r*, *s*, empty sporangia. *a*–*f*, *h*–*m*, ×945; *n*–*p*, *r*, *s*, ×765; *g*, ×450; *q*, ×720.

sporangia, thirty to sixty in large ones. In a few mature sporangia a dully refractive area (Fig. 2*e*, *f*) was seen just below the apical spine, which probably marked the region of dehiscence. On dehiscence of the larger sporangia, part of the sporangial wall between the apical spine and first whorl of spines deliquesces to form a pore. In the smaller sporangia this dehiscence pore is more median in position (Fig. 2*j*). A dehiscence papilla is never developed. The contents of the sporangium emerge to form a mass outside; although no definite vesicle was observed continuous with the sporangium, the zoospore mass is held together by some invisible substance. If this mass is moved while under a cover-slip, it remains connected with the sporangium. After a minute or so the individual zoospores are delimited, although still entangled by their flagella. They soon become freed from one another and swim away individually with a rapid darting movement. The zoospores are spherical, 4–5 μ in diameter, with a conspicuous posterior oil globule, above which is a clearer area containing a refractive granule; the remainder of the protoplasm is coarsely granular (Fig. 2*h*).

In spite of the great range of thallus structure it seems that only one species is concerned, since intermediate stages from small thalli with only an apical spine to the thalli with four spine whorls are to be found. A few specimens of this chytrid were parasitized by an unidentified species of the same order (Fig. 2*n*–*p*). Although the sporangia resemble *Septosperma anomalum* (Couch) Whiffen (which has recently been found by me on *Chytriomyces tabellariae* (Schröter) Canter in a nearby bog), it cannot be definitely assigned to this species until the resting spore is discovered.

Phlyctochytrium mucronatum more especially resembles the dentigerate members of this genus (see Sparrow, 1943, pp. 229–34), e.g.: *P. planicorne* Atkinson, *P. zygnematis* (Rosen) Schroeter, *P. quadricorne* (de Bary) Schroeter, *P. bullatum* Sparrow, *P. urceolare* Sparrow, *P. dentiferum* Sparrow and *P. aureliae* Ajello (1945). They have all been observed as saprophytes; in the first three and *P. aureliae* the zoospores are described as emerging in a mass from the sporangium and in none is the resting spore known. However, the wide variation in thallus structure exhibited by *P. mucronatum* has not been recorded for the other species. The large sporangia most closely resemble those of *P. aureliae* except that in the latter the teeth are scattered over the surface in an apparently haphazard fashion and by proliferation of their apices may become setigerous or thread-like. Again in *P. aureliae* the zoospores emerge by way of a rupture in the sporangial wall as in *P. mucronatum*. In other *Phlyctochytrium* spp. an apical dehiscence papilla, surrounded by a collarette of teeth is formed. The characteristic apical spine clearly distinguishes *P. mucronatum* from *P. aureliae*. In its saprophytic habit, apophysis, extensive rhizoidal system and emergence of the zoospores in a mass, *P. mucronatum* shows a superficial resemblance to the exuviaceous chytrids, especially the genus *Asterophlyctis*. As mentioned earlier (p. 236), until the resting spores are known the exact affinities of this organism remain obscure, but there is little doubt that the chytrid here described represents a new species. The following diagnosis is suggested.

Phlyctochytrium mucronatum sp.nov.

Thallus epibioticus, monocentricus, e sporangio, apophysi et rhizoideis extensis compositus. Sporangia subglobosa, 5·7–31 μ diam. (spinulis inclusis) spinulam singulam pyramidatam apicalem 1·4–5·2 μ longam, basi 4·3 μ latam, et spinulas laterales (in sporangiis parvis deficientes) simplices vel furcatas in verticillis 1–4 dispositas gerentia, poro laterali dehiscentia, cytoplasma integra emergente. Zoosporae sphaericae 4–5 μ diam., intus crasse granulosae, postice globula oleosa et supra spatio clariore et granulo nigro praeditae. Apophysis sphaerica, 1·3–3·5 μ diam. Rhizoidea ramosa, axibus principalibus 1–3 ex apophysi oriundis, saepe dichotome ramosis, apices versus angustatis, ad 85 μ longis. Sporae perdurantes non visae.

Hab. Saprophyticus, in *Closterio pritchardiano* Arch. et *C. costato* Corda, Clay Pond et Blelham Bog, Wray Castle, Anglia, 1946.

Phlyctochytrium mucronatum n.sp.

Thallus epibiotic, monocentric, consisting of a sporangium, apophysis and extensive rhizoidal system. Sporangia more or less spherical 5·7–31 μ in diameter (including spines), with a single pyramidal apical spine 1·4–5·2 μ long × 0·9–4·3 μ broad at the base and with one to four whorls of lateral simple, or Y-shaped spines (absent in very small sporangia). Sporangium dehiscing by a lateral pore; contents emerging in an undifferentiated mass continuous with the sporangium. Zoospores spherical, 4–5 μ in diameter, with a posterior oil globule, above which is a clearer area with a black granule; remainder of the protoplasm is coarsely granular. Apophysis spherical 1·3–3·5 μ in diameter. Rhizoidal system one to three main axes leaving the apophysis, often branching dichotomously and tapering towards their extremities, up to 85 μ long. Resting spores not observed.

Saprophytic on *Closterium pritchardianum* Arch. and *C. costatum* Corda, the Clay Pond, and Blelham Bog, Wray Castle, England, 1946.

My thanks are due to Miss E. M. Wakefield of Kew for the Latin translation and to Prof. C. T. Ingold for reading the manuscript.

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LEAF DISEASES OF *ANEMONE CORONARIA* IN CORNWALL

By P. H. GREGORY, *Rothamsted Experimental Station, Harpenden, Herts.*

(With Plate XX and 2 Text-figures)

In the ten years before 1940 the winter production in the open air of cut flowers of *Anemone coronaria* increased rapidly in south-west England. The crop is grown either from seed sown in March or April, or from 1–3 cm. corms, usually of Dutch origin, planted in July (Johnstone & Walbank, 1938). The notes which follow describe the leaf diseases of anemone observed during the years 1935–40.

Winter Browning. The most important anemone disease in the south-west has been named Winter Browning by Beaumont and Staniland (1935), who described the course of the disease and discussed possible causes. It usually appears in the winter months and may be associated with root decay, a purplish tint to the leaf, and decay of the leaf margin. The corm is normally unaffected, but the flower crop is seriously reduced, and flowers already formed are small and brown at the edges. The disease spreads to form large patches which may eventually cover the entire field. In moist dull weather diseased tissues may become covered with a copious growth of *Botrytis cinerea*, but inoculations with strains isolated from diseased plants have failed to reproduce the symptoms on healthy plants. The cause of winter browning is not yet known, but the possibility of it being caused by a virus, such as spotted wilt or cucumber mosaic virus, needs to be investigated. Anemones grown from seed usually remain free from the disease, while it often devastates fields planted with corms.

Cluster Cup Rust (*Puccinia pruni-spinosae* Pers.) rarely occurs in commercial plantings of *Anemone coronaria*, which is grown as an annual crop. When beds are left for a second year, however, plants bearing hypertrophied leaves with pycnidia and aecidia of the rust often develop in spring. *A. fulgens* grown as a perennial from corms imported from France may show up to 50 % of diseased plants. Affected shoots flower poorly, if at all. A corm may be only partly infected and produce both normal and hypertrophied shoots. Domestic and wild plum are commonly attacked in the south-west.

Powdery mildew (*Oidium* sp.) was first recorded at Penzance on 17 October 1935 on anemones grown from corms. The attack was slight and the fungus appeared as a thin white, powdery layer in small patches on otherwise normal leaves, petioles and flower stalks. Microscopic examination showed numerous superficial radiating mycelia on apparently healthy parts of the epidermis, producing abundant spores of *Oidium*. The perithecial stage has not yet been recorded on *Anemone coronaria* and the identity of the fungus must remain in doubt. Beaumont (1938) has indicated that it is probably *Erysiphe polygoni* DC. Traces of the mildew were found each autumn, but it became severe only in the middle of October 1937 when six acres of St Brigid and anemone de Caen at St Erth, Cornwall were badly affected. The plants, grown from corms from various sources on a north-west and west slope and obviously suffering from the effects of drought, were so heavily infected with the mildew that the field looked as though it had been lightly dusted with lime. The characteristic aromatic odour of powdery mildew was obvious. The conidiophores were most numerous on the laminae of the older leaves, but were also present on the younger leaves. The flowers were poor with short stems. At the same date the disease was severe on anemone de Caen grown from seed at St Hilary. In September this field was yellow from drought, but by the middle of October it had recovered and appeared a healthy green except where whitened by the mildew. By December new leaves had been produced which remained free from attack, and the fungus could be found only after a search on the older leaves.

DOWNY MILDEW (*Plasmopara pygmaea* (Unger) Schroet.) was first reported on *Anemone coronaria* in Cornwall by Beaumont (1938). It was observed at the end of September 1937 on a field of anemone de Caen near Liskeard, where the foliage of a patch three-quarters of an acre in extent became blackened and died in about four days. Conidiophores of a downy mildew were apparent with a hand-lens, maximum conidial production being from stomata on portions of the green leaf immediately outside the blackened zone. The fungus was readily identified as *Plasmopara pygmaea* (Text-fig. 1). Oospores were formed in the discoloured tissues of the leaf. By the beginning of December the crop had recovered, no *Plasmopara* could be found and the plants were producing flowers again.

DOWNY MILDEW (*Peronospora ficariae* Tul.). A second downy mildew on *Anemone coronaria* and one which is commoner than *Plasmopara pygmaea* though usually much less conspicuous, may be referred provisionally to *Peronospora ficariae* Tul. Its occurrence has been reported by Moore (1943), and it is apparently the first *Peronospora* to be found on any species of anemone. It was first noticed in September 1935 during microscopic examination of apparently healthy leaves of anemone de Caen collected at Penzance. Many leaves were free from the fungus, but on others isolated sporangiophores could be found protruding through a few of the stomata. Often a large area of leaf had to be searched before a single specimen could be found. These sparsely distributed sporangiophores were never, in this collection, associated with visible lesions, and the fungus could not be seen with the naked eye. Further collections from the same field in the next few weeks yielded some more material, but it disappeared during the winter. It was not possible to ascertain whether infected leaves subsequently died. Early in December 1936 the disease appeared in a more severe form in a garden at Newton Abbot where four beds of *Anemone coronaria* were blackened. The leaves had blackish necrotic marginal lesions and were covered with conidiophores of the downy mildew visible to the naked eye or with a hand-lens. The affected leaves were rolled upwards, thus bringing most of the sporangiophores, which protruded through the stomata on the lower surface of the leaf, into an erect position (Pl. XX, fig. 1). The bracts were also affected. Sporangia germinated by zoospores, a character associated with *Plasmopara*, rather than *Peronospora*, but germination by zoospores has been reported in *P. spinaciae* by Eriksson (1918). The conidiophores (Text-fig. 2), however, were entirely different from *Plasmopara* and resembled a figure of *Peronospora ranunculi* Gäumann (1923), a species which is also a member of what Gäumann considers to be the *Formenkreis P. ficariae* Tul. The sporangia became swollen in water and before germination measured 28–39 × 23–30 μ. Oospores were not seen in any of the material examined. It is possible that *P. ficariae* is more widespread on anemones and does more damage than these records suggest. Further investigation may show how often blackening of anemone foliage is the sequel to production of a crop of conidia and disappearance of the conidiophores.

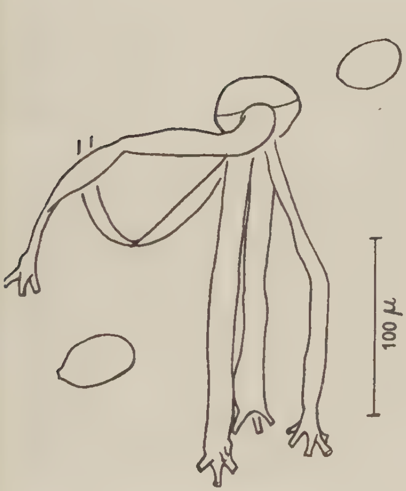

Fig. 1

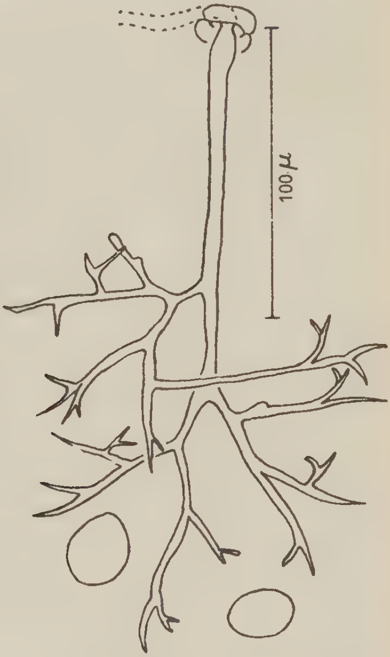

Fig. 2

Text-fig. 1. *Plasmopara pygmaea* (Unger) Schroet. Camera lucida drawing of conidiophores and conidia from leaf of *Anemone coronaria*, Liskeard, Cornwall, 27 September 1937.

Text-fig. 2. *Peronospora ficariae* Tul. Camera lucida drawing of conidiophores and conidia on leaf of *Anemone coronaria*, Penzance, 11 October 1935.

Black leaf spot (*Septoria anemones* Desm. var. *coronariae* Grove). This disease occurs occasionally in autumn, forming conspicuous dark brown or black spots on mature leaves, usually near the margins (Pl. XX, fig. 2). The diseased tissue is dry and sunken and deeply discoloured with a brownish black pigment. The blackened area is sharply demarcated from the surrounding healthy green area. Examination of the blackened area with a hand-lens shows the presence of numerous black pycnidia from which masses of spores are exuded in cirri.

The disease was first noted on a small plot of anemones at Gulval, near Penzance, Cornwall, on 16 September 1936. The spots were particularly numerous where the plants were shaded by a *Pittosporum* hedge. The ground had previously carried a crop of lettuce and had subsequently received a dressing of farmyard manure and fish manure, before being planted with 1–2 cm. anemone corms at the end of May. Several varieties were affected including de Caen, St Brigid and Hollandia. The disease spread over the plot during October and November and persisted through the winter. During March 1937 the foliage on the affected beds was killed by strong dry winds and the new foliage which grew later remained free from the disease. The disease was not observed on the 1937–8 crop, but reappeared within a few hundred yards of the first outbreak in December 1938 on anemones grown from seed sown the previous March.

Septoria anemones Desm., which is said to be common on our wild *Anemone nemorosa*, was described by Grove (1935–7) as having 'spots dry, at length brown...not bordered, sometimes hardly different in colour from the rest of the leaf, greenish or brown, paler in the centre'. The spores measure 20–22 × 1–1·25 μ.

Microscopically the fungus on *A. coronaria* resembles *Septoria anemones* Desm. except that the spores are longer, measuring 30–42 μ in the Cornish material. The late W. B. Grove, to whom specimens were submitted, stated that the length of the spores was not sufficient to differentiate the specimens on *Anemone coronaria*, but he considered that the black colour of the spots justified making a new variety. He accordingly described *Septoria anemones* var. *coronariae* as producing 'spots dark-brown or almost black, large, well-defined, mostly marginal, irregular in form, but not bordered with another colour. On leaves of *Anemone coronaria*, Penzance (Gregory). Oct.' (Grove, 1935–7). The leaves of *A. coronaria* readily turn dark brown on wounding or death from any other cause, and it is possible that the colour of the spots is in part a character of the host.

The fungus grows readily and produces spores on artificial media (glucose-peptone and glucose-asparagin agar).

Specimens and slides of the *Oidium* sp., *Plasmopara pygmaea*, *Peronospora ficariae* and co-type material of *Septoria anemones* var. *coronariae* on *Anemone coronaria* have been deposited in the Herbarium of the Ministry of Agriculture, Plant Pathology Laboratory, Harpenden.

I carried out this work under a scheme financed jointly by the Great Western Railway Co. through the Cornwall Farmers' Union and the Ministry of Agriculture and Fisheries, upon the recommendation of the Agricultural Research Council, to whom grateful acknowledgements are made. Thanks are also due to Mr A. Beaumont and Mr W. C. Moore for help given in the course of the work.

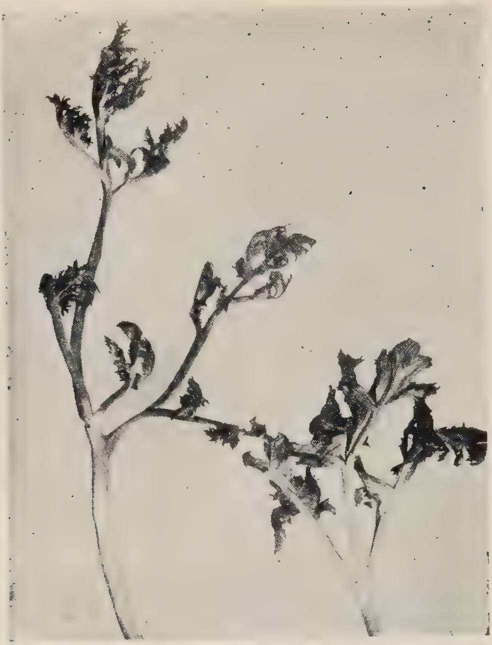

Fig. 1

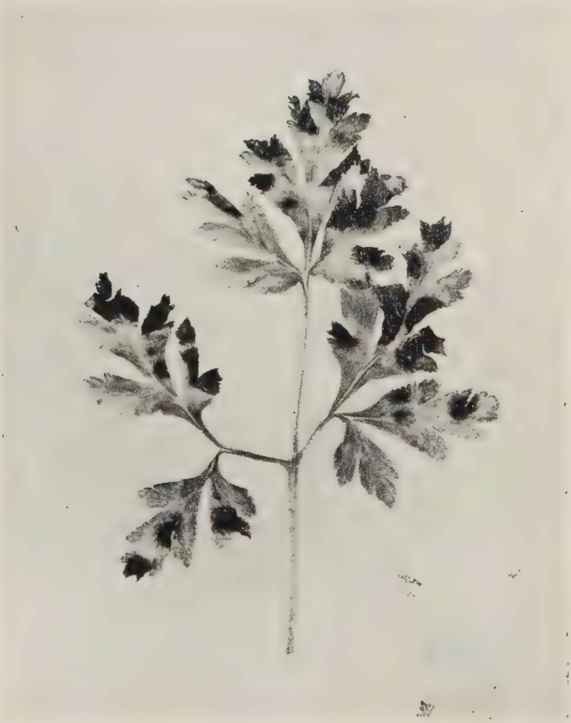

Fig. 2

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EXPLANATION OF PLATE XX

Fig. 1. Leaf of *Anemone coronaria* attacked by *Peronospora ficariae* Tul., Newton Abbot, December 1936.

Fig. 2. Leaf of *Anemone coronaria* attacked by *Septoria anemones* var. *coronariae* Grove, Penzance, 16 September 1936.

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TETRAPLOA

By M. B. ELLIS, *Commonwealth Mycological Institute, Kew*

(With 2 Text-figures)

Up to the present time five species of *Tetraploa* have been described: *T. aristata* Berk. & Br. (the type species), *T. divergens* Tracy & Earle, *T. ellisii* Cooke, *T. muscicola* Gonz. Frag., and *T. scabra* Harkn. Only brief descriptions of these fungi have been published. In this paper *T. aristata* and *T. ellisii* are redescribed and an account is given, for the first time, of cultural characters and the development of the spores in the former; notes on the other species are appended.

1. *TETRAPLOA ARISTATA* Berk. & Br.

The original description of *T. aristata*, drawn up by Berkeley and Broome (1850), was based on material on grass collected at Westhay Woods, King's Cliffe, Northants. Since 1850, surprisingly few records of this easily recognized fungus have been made, in spite of the fact that it occurs throughout the year on a number of grasses, sedges and other plants, and has a wide geographical distribution. In Great Britain it has been recorded on *Phalaris arundinacea* (Stevenson & Trail, 1885), on an unnamed host (Bucknall, 1886), on grass, probably *Poa pratensis*, cut and thrown into a heap, and on living roots of grass (Bayliss Elliott & Stansfield, 1923), on *Cladium mariscus* (Ellis, 1941 and B.M.S. Foray Report, 1947), on *Phragmites communis* (Ellis, 1941), on *Angelica sylvestris*, *Heracleum sphondylium* and *Pteridium aquilinum* (E. A. Ellis in litt.). Thirty collections were made in this country between 1940 and 1947 on dead leaves and stems of *Ammophila arenaria*, *Carex paniculata*, *Cladium mariscus*, *Cortaderia selloana*, *Cyperus longus*, *Phragmites communis* and wheat stubble. The spring gatherings showed large numbers of young developing spores; in the late summer, autumn and winter gatherings, mature spores predominated. Outside Great Britain the fungus has been recorded on *Ammophila arenaria* in Holland and on *Avena pratensis* [*Helicotrichon pratense*] in Germany (Lindau, 1908), on *Bambusa* in China (Wei & Hwang, 1924–37), on *Festuca* in Italy (Ferraris, 1912), on *Gynerium argenteum* [*Cortaderia selloana*] in Eire (Pim, 1884), on *Saccharum officinarum* in Alabama, Barbados, Cuba, the Dominican Republic and Puerto Rico (Stevenson & Rands, 1938), on straw in Denmark (Lind, 1913), on wheat stubble in Holland (Van de Laar, 1931) and on an unnamed host in Japan (Shirai & Hara, 1927). Two recent gatherings from Africa have been examined, one on *Anadelphia leptocoma* collected in Sierra Leone by F. C. Deighton, and the other on *Cymbopogon afronardus* collected in Uganda by C. G. Hansford.

Colonies of the fungus on the host are brown or dark greyish brown in colour; they are either very small and localized, or else they form an irregular mat spreading right round the leaf or stem and four to five centimetres along it. The mycelium is superficial and is made up of a weft of branched and anastomosing septate hyphae 1·5–3 μ in diameter, hyaline to pale yellowish brown, with smooth to verruculose walls (Fig. 1 S).

Two kinds of conidia are formed directly on the mycelium: (*a*) large ones with setose appendages about the length of the spore (Fig. 1 Q, R), and (*b*) small conidia with very long appendages (Fig. 1 T, U). Probably the larger conidia only were observed by Berkeley and Broome who likened them aptly to 'the achaenium of some composite plant'. Each conidium arises as a small bud with deeply staining cell contents (Fig. 1 A–D and H); this bud swells and becomes divided into two by a vertical wall (Fig. 1 E, I); each of the two cells thus formed is divided again by a second vertical wall at right angles to the first (Fig. 1 F, J). No further walls are laid down in a vertical plane, but each of the four initial cells swells, elongates, and as a rule becomes divided by horizontal walls into a column of from two to six cells (Fig. 1 G, K and L). In mature spores there are shallow furrows between the columns of cells which are formed independently and tend to diverge from one another apically. The columns are sometimes of unequal length; in each, the terminal cell puts out a process (Fig. 1 M–O) which elongates and becomes a septate setose appendage. Cotton blue in lactic acid stains the rounded contents of each cell and shows up strands of protoplasm linking them together. There is apparently protoplasmic continuity by pores between the four initial cells, the individual cells in each column, the terminal cells and the setose appendages (Fig 1 R). Mature conidia are brown, thick-walled and verrucose; the appendages are smooth, brown at the base and almost hyaline at the tips. Measurements and structural details of the two kinds of conidia are tabulated below:

	Average of twenty conidia (μ)	
	(*a*)	(*b*)
Body of conidium:		
Length	25–39 (31·8)	8–18 (12)
Diameter at base	7–14 (10·7)	7–12 (9·5)
Maximum diameter	14–29 (20·6)	9–16 (12·3)
Number of cells in each column	3–6	1–2
Setose appendages:		
Length	12–80 (36)	80–330 (218)
Diameter at base	4·5–8 (5·97)	3–6 (4·97)
Diameter at apex	2–3·5 (2·45)	1–2 (1·97)
Number of septa	1–6	3–12

Tetraploa aristata is easily grown on potato-dextrose agar. Germ tubes are produced from the body of the conidium and from the ends of the setae. Spores germinated on 27 August 1947 and grown at room temperature formed by 8 September greyish brown, freely sporing, colonies 12–14 mm. in diameter; the conidia formed did not differ in any way from those produced on natural substrata. The septate, branched mycelium, composed of hyphae 2–3 μ in diameter, appeared pale yellowish brown by transmitted light and was seen to be verruculose.

The spores of this fungus with their long appendages resemble those of certain aquatic hyphomycetes and it is thought that water aids in their

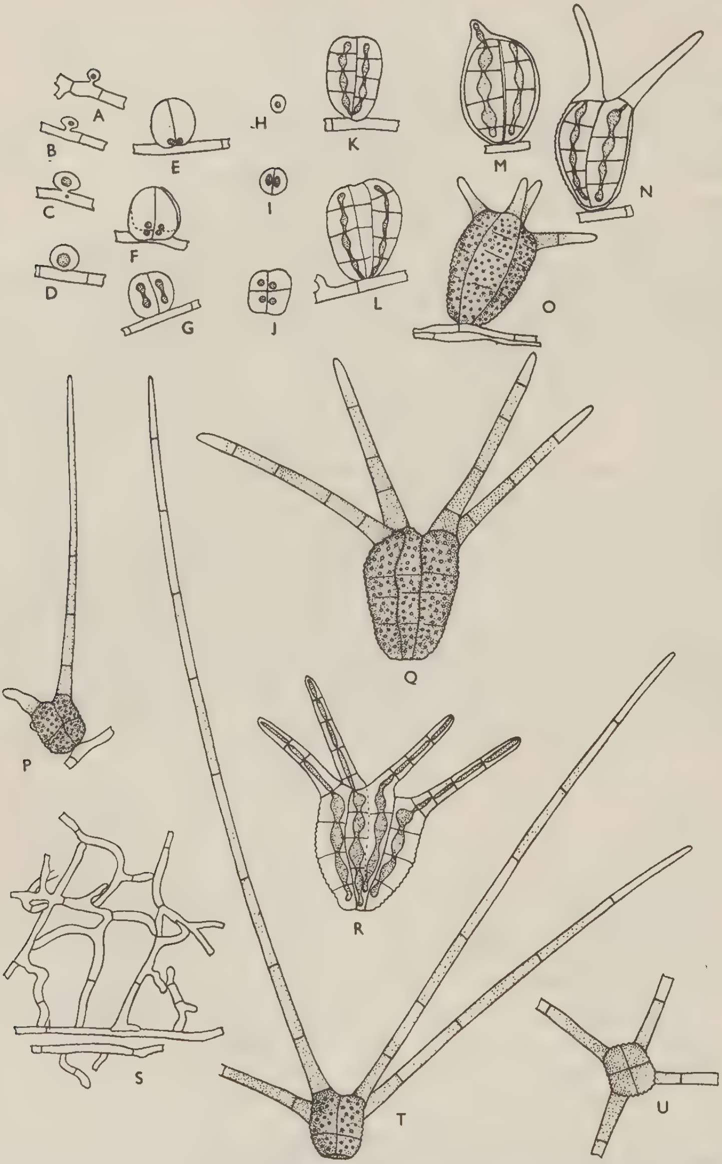

Fig. 1. *Tetraploa aristata*. A–O, development of *a* spores; P, young *b* spore. Q, mature *a* spore; R, *a* spore stained to show protoplasmic linkage by pores; S, mycelium; T, lateral view of *b* spore; U, end view of *b* spore. (All × 500.)

dispersal. When an infected leaf or stem is covered with a thin film of water a number of spores break off and float to the surface; on tilting they move down with the flow. Spores mechanically detached from the substratum and placed in a 250 c.c. beaker full of water floated for several hours; when submerged they sank at the rate of 1·5 cm. per minute.

T. aristata is usually found on leaf bases and on stems just above the soil although it also grows at a much higher level on dead standing leaves of *Cladium* at Wheatfen Broad. Most of the recent collections of this fungus have been made on marshes and fens subjected to periodic flooding and here it is probable that flood water plays at least some part in spore dispersal. Spores of a number of marsh fungi, including *Tetraploa aristata*, have been found floating on the surface of dykes.

Specimens examined:

On *Ammophila arenaria*, Scratby, Norfolk, 26 August 1945, E. A. Ellis (Herb. I.M.I. No. 23223).

On *Anadelphia leptocora*, Kenema, Sierra Leone, 6 July 1937, F. C. Deighton (Herb. I.M.I. No. 22000).

On *Carex paniculata*, Wheatfen Broad, Norfolk, 27 December 1946, E. A. Ellis (Herb. I.M.I. No. 10523).

On *Cladium mariscus*, Wheatfen Broad, Norfolk, 1940–7, E. A. and M. B. Ellis: January (Herb. I.M.I. No. 10323), March (Herb. I.M.I. Nos. 21397 and 21398*a*), April (Herb. I.M.I. Nos. 14875*h*, 16534*b*, 21400 and 21401*a*), May (Herb. I.M.I. Nos. 8998*a*, 15331, 15393*c* and 15413*f*), June (Herb. I.M.I. Nos. 8999 and 21440), August (Herb. I.M.I. Nos. 16670 and 17342), September (Herb. I.M.I. No. 21441), November (Herb. I.M.I. Nos. 8911*a*, 8912 and 21442), December (Herb. I.M.I. Nos. 10888, 21149*c* and 21276*b*); Derby Fen, Grimston, West Norfolk, 26 August 1945, E. A. Ellis.

On *Cortaderia selloana*, Perranzabuloe, West Cornwall, 27 May 1947, F. Rilstone (Herb. I.M.I. No. 15540*a*).

On *Cymbopogon afronardus*, Kisube, Entebbe, Uganda, October 1944, C. G. Hansford (Herb. I.M.I. No. 178).

On *Cyperus longus*, Grand Mare, Guernsey, Channel Islands, 6 July 1939, E. A. Ellis (Herb. I.M.I. No. 21444).

[On grass], Westhay [Woods, King's Cliffe, Northants]; ex Herb. Berk. 1879, in Herb. R.B.G. Kew (slide filed as Herb. I.M.I. No. 17841). Type.

[On grass], labelled *Tetraploa aristata* in C. E. Broome's handwriting; ex Herb. R.B.G. Kew (slide filed as Herb. I.M.I. No. 17842).

On *Phragmites communis*, Wheatfen Broad, Norfolk, April, E. A. and M. B. Ellis (Herb. I.M.I. Nos. 14514*c* and 21443).

On wheat stubble, Newton St Cyres, Devonshire, 18 September 1947, M. B. Ellis (Herb. I.M.I. No. 17534).

2. *TETRAPLOA ELLISII* COOKE

The first description of *T. ellisii* Cooke, published by Cooke and Ellis (1879), was based on material on old stalks of *Zea mays* collected at Newfield, New Jersey, in 1878. The fungus appears to have been recorded only from North America where it occurs on the type host and on *Arundinaria tecta*. The colonies are brown and cover an area of 3 sq.cm. or more. The superficial mycelium is composed of hyaline to pale brown, branched septate hyphae, $2{\cdot}5$–$4\,\mu$ in diameter, with verruculose walls. Only one type of conidium has been seen in which the appendages are on the whole longer than those of the *a* spores and shorter than those of the *b* spores of

Tetraploa aristata (Fig. 2). The body of the spore is brown, smooth or verrucose (often verrucose only at the base) and made up of three or four columns of cells each of which ends in a gradually attenuated, smooth-walled, septate appendage. Measurements and structural details of the conidia are given below:

	Average of ten conidia (μ)
Body of conidium:	
Length	30–51 (39)
Diameter at base	10–18 (13)
Maximum diameter	15–26 (20·6)
Number of cells in each column	4–8
Setose appendages:	
Length	24–178 (97)
Diameter at base	7–10 (8·7)
Diameter at apex	2–4 (2·8)
Number of septa	2–18

Specimen examined:

On old stalks of *Zea mays*, Newfield, New Jersey, U.S.A., October 1878, J. B. Ellis; ex. Herb. R.B.G. Kew (slide filed as Herb. I.M.I. No. 8783). Type.

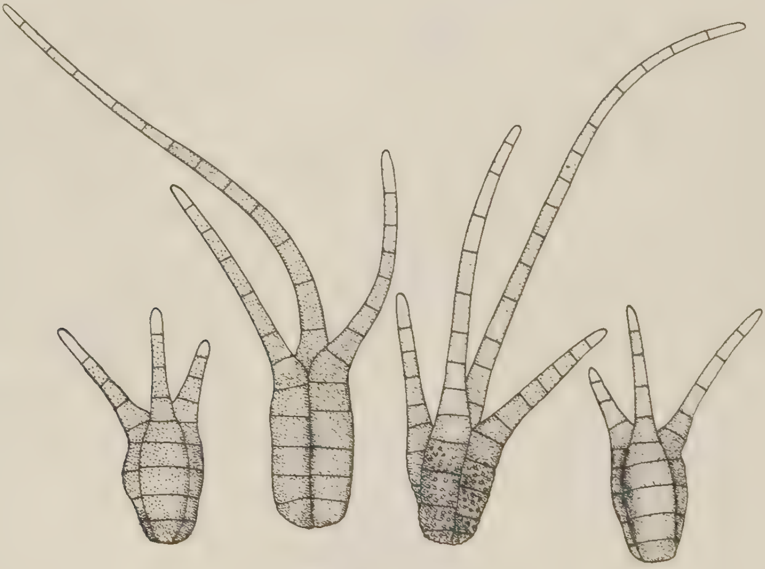

Fig. 2. *Tetraploa ellisii*, mature spores. (× 500.)

3. Other species of *Tetraploa*

Little has been written about the other three species of *Tetraploa* and I have not seen specimens of them. *T. divergens* was found growing on *Panicum agrostidiforme* [*P. laxum*] at Starkville, Miss., U.S.A., October 1894 and was described by Tracy and Earle (1895). According to their description the spores are about the same size as the *b* spores of *Tetraploa aristata* (12–14 × 8–9 μ); the appendages however are very short (4–5 μ) and without septa; there are usually three cells in each of the four columns. *T. scabra* is stated to have oval brown spores, 30 × 50 μ, studded with minute papillae; the widely diverging septate setae are often ten times the length of the spore (Harkness, 1885); it was found on *Scirpus* at San Francisco, U.S.A. *Tetraploa muscicola* has been recorded only from Spain (Gonzalez Fragoso, 1916) on the liverworts *Aneura multifida* and *Lophozia quinquedentata*. From their descriptions these species appear to be distinct from *Tetraploa aristata* and *T. ellisii*.

SUMMARY

Tetraploa aristata is redescribed and an account given of its development, cultural characters, host range, spore dispersal and geographical distribution.

A description of *T. ellisii*, based on type material, is given; notes are made on three other species of *Tetraploa*.

I wish to thank Dr S. P. Wiltshire, Mr E. W. Mason, Dr G. R. Bisby and my brother Mr E. A. Ellis for kindly help and criticism.

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A BACTERIAL LEAF AND FLOWER DISEASE OF *ZINNIA* IN SOUTHERN RHODESIA

By J. C. HOPKINS and W. J. DOWSON

(With Plate XXI)

An angular leaf spot of *Zinnia* was first observed in Southern Rhodesia about twelve years ago but was not reported again until March 1945, when both leaves and flowers of a large bed of plants growing at the Plant Pathological Laboratory in Salisbury were severely affected. Reddish and violet coloured varieties were more severely attacked than were those with yellow flowers.

Investigation showed that the disease was due to a bacterial pathogen, and the symptoms suggest that it may be the same as the *Zinnia* disease briefly described by Nannizzi (1929) in Italy. Nannizzi observed that the lesions were associated with bacteria often accompanied by *Trichoderma*, but he did not carry out any infection experiments with the bacteria, which he stated were spore-formers.

In Southern Rhodesia the disease first appears on the leaves as diffuse, yellowish, translucent, circular spots, 1–2 mm. in diameter. Under conditions of intermittent rainfall the spots slowly enlarge to about 5 mm. across and small reddish brown spots develop in the centres of the larger lesions, which continue to increase and become angular in shape. Each lesion is surrounded by a narrow margin of yellowish tissue, and if numerous, the lesions coalesce into rectangular areas 0·5–1·0 cm. long. Linear lesions may also form along the veins and develop into large dead areas involving most of the lamina. Generally, however, individual spots can still be recognized in the dead patches as angular areas varying in colour from dark brown to greyish white.

When atmospheric humidity is very high, there is a rapid expansion of the initial yellow spots. The dead areas quickly increase in size, coalesce, and if the humidity remains high, develop into ragged patches of decaying tissue. Under such conditions the disease may extend to the flowers and produce small brown spots on the ray florets. If infection is severe, the heads are seriously disfigured and may decay completely.

A yellow and a white bacterium were obtained from the lesions on the leaves and flowers in mixed culture, and were isolated in pure culture. Suspensions of each in sterilized water were atomized on to young *Zinnia* plants, but infection was obtained only with the yellow bacterium. Control plants remained healthy. The yellow bacterium was re-isolated and the infection experiments were repeated twice with positive results. Two other members of the Compositae, lettuce and burdock (*Arctium lappa*), were also inoculated in the same way, but neither was infected. Of the varieties of

Zinnia so far tested, Purple Prince, Brightness, Royal Purple and Lavender Queen are highly susceptible, the last-named being attacked in the stem, which may collapse. Yellow-flowered varieties develop mainly marginal infection, while red seem to be intermediate.

The *Zinnia* organism is a Gram-negative, rod-shaped bacterium with rounded ends and is provided with a single, long, polar flagellum. Yellow colonies are formed on most solid media. A honey coloured, slimy, abundant growth is produced on sterilized potato. Litmus milk becomes slightly alkaline and is peptonized without reduction of the litmus. Salicin is not attacked. The foregoing characters are those of the genus *Xanthomonas* (Dowson, 1939).

Two species of *Xanthomonas* are known to attack members of the Compositae: one, *X. vitians*, causes a wilting disease of lettuce in America (Brown, 1918); the other, *X. nigromaculans*, produces black spots on the leaves of *Arctium lappa* in Japan (Takimoto, 1927). The brief description of the last named available to us (Elliott, 1930) corresponds with that of *Zinnia* here recorded, and it is possible that the *Zinnia* bacterium may be a '*forma specialis*' of Takimoto's organism. The physiological characters and biochemical activities of *Xanthomonas nigromaculans* have not been fully investigated, and until these are known, or a culture is available, it is not possible to come to a definite decision as to whether or not the *Zinnia* pathogen is identical with that isolated from *Arctium*.

In view of the negative results obtained from the infection experiments with *Arctium*, of the fact that species of *Xanthomonas* are, in general, confined to a single genus of host plants, and also of the desirability of assigning a definite name to the *Zinnia* pathogen, we suggest that it should be regarded provisionally as a '*forma specialis*' of *Xanthomonas nigromaculans* (Takimoto) Dowson.

The following is a brief technical description of the *Zinnia* pathogen:

Xanthomonas nigromaculans forma specialis **zinniae** f. n.sp.

Gram-negative rods with rounded ends, provided with one polar flagellum. Forming yellow colonies on meat-infusion or potato-glucose agar, and abundant, slimy, honey-coloured growth on sterilized potato. Hydrolyses starch. Does not attack salicin, reduce nitrates, or form ammonia from peptone. Produces hydrogen sulphide from peptone and liquefies gelatin. Slowly digests litmus milk which is rendered slightly alkaline, but does not reduce litmus. Forms acid in glucose, sucrose, maltose and lactose in peptone-free media.

Pathogenic in leaves and flowers of *Zinnia elegans*, forming yellow spots becoming brown.

Type locality: Salisbury, Southern Rhodesia.

Distribution: Southern Rhodesia and possibly Italy.

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EXPLANATION OF PLATE XXI

Fig. 1. Naturally infected leaves. × 1.
Fig. 2. Artificially infected leaves. × 1.

(*Accepted for publication* 5 *March* 1948)

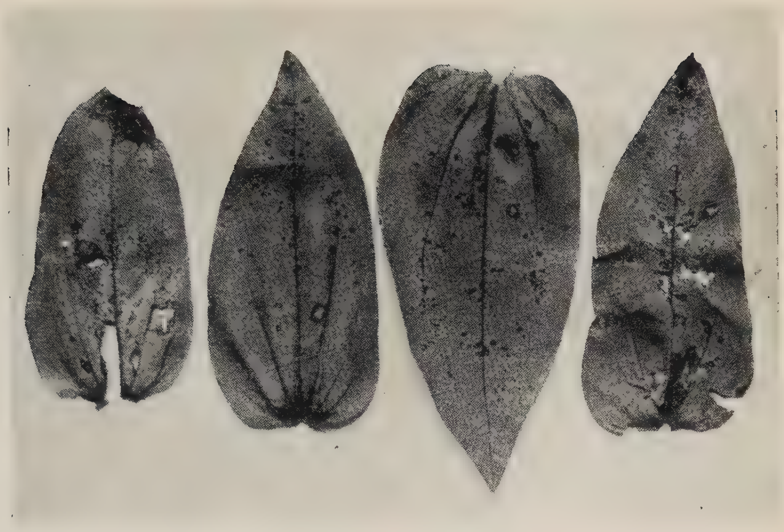

Fig. 1

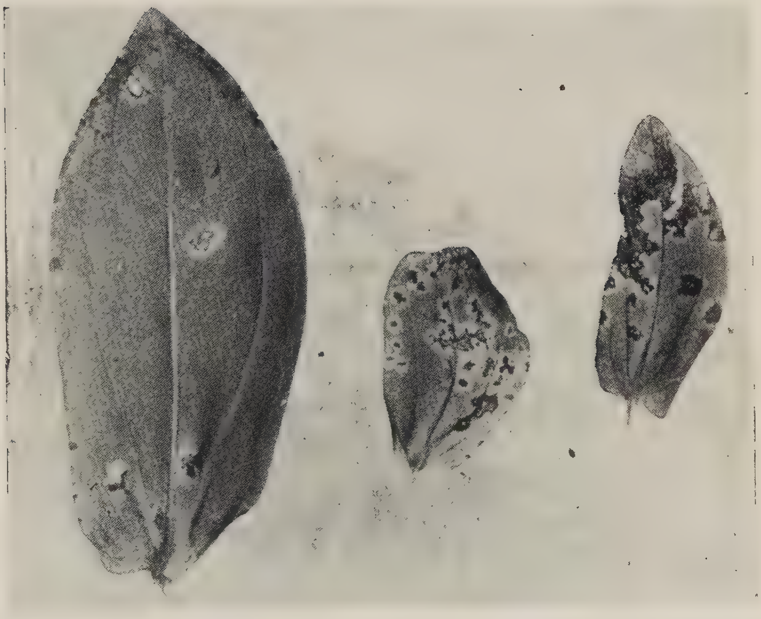

Fig. 2

THE GLADIOLUS SMUT

By G. C. AINSWORTH
London School of Hygiene and Tropical Medicine

During recent years the status of the gladiolus smut has been a matter of uncertainty. Dodge and Laskaris (1941), from their study of a 'Smut' disease of gladiolus in New York, concluded that the gladiolus disease previously reported from both Europe and North America as smut was not caused by one of the Ustilaginales, but by a species of *Papulaspora* which, without examining European material, they named *P. gladioli* (Requien) Dodge & Laskaris. The next year Hotson (1942*a*) described the results of a comparative study of material provided by Dodge and English material, from which he concluded there were two distinct diseases: one caused by a species of *Papulaspora* characterized by bulbils, 24–64 μ [28–64 μ in the histogram in his fig. 3] diam., with the average at 44 μ, composed of 2–8 central cells surrounded by a cortex of one or more layers of cells, the other by a species of *Urocystis* characterized by spore-balls, 14–23 μ [12–32 μ in the histogram] diam., each composed of one or two central cells surrounded by a single layer of cortical cells. He proposed the first fungus as a new species under the name *P. gladioli* Hotson (a later homonym for *P. gladioli* (Req.) Dodge & Laskaris and therefore invalid) and adopted *Urocystis gladioli* (Req.) Smith for the second. Six months later Hotson (1942*b*) described three more species of *Papulaspora* associated with decay in gladiolus corms and in the same paper *P. dodgei* Conners was proposed to replace '*P. gladioli* Hotson'. The bulbils of all these four species are appreciably larger than the spore-balls of the smut (see Hotson, 1942*b*, fig. 2).

In the autumn of 1941 I compared a specimen of '*P. gladioli*', kindly supplied by Dr B. O. Dodge, with the English collection (Herb. Path. Lab., No. 673) of which Hotson examined a part, and concluded, as did Hotson, that two species of fungi were involved. Search for the necessary type specimens could not be made under wartime conditions and only recently has the evidence needed to establish a name for the smut been assembled.

Urocystis gladioli W. G. Smith

The usually accepted designation for the gladiolus smut is *U. gladioli* (Requien) W. G. Smith, the name under which the species was compiled by De Toni in Saccardo's *Sylloge* (VII, p. 518, 1888). W. G. Smith (1876) did not, however, transfer Requien's species to *Urocystis*. He made no mention of the earlier species and wrote (p. 421): 'This new species I propose naming *Urocystis Gladioli*, and it may be characterized as follows: Sori (or clusters of spores in blisters) obliterated or effused, spores large, compound, consisting of from three to six inner brown cells, and a larger, indefinite number of nearly transparent outer cells, both series of cells being fertile. Habitat—on and in the corms and scapes of Gladioli. (See fig. 84, enlarged 200 diam., and A, B, fig. 85, enlarged 1000 diam.)'

Search in the Herbaria of the British Museum (Natural History) and of the Royal Botanic Gardens, Kew, has failed to locate a type specimen, although at Kew there is a fragment of gladiolus corm labelled '*Urocystis gladioli* W. G. Smith' (without host, locality, or date) in W. G. Smith's handwriting. Careful examination of this specimen disclosed no spores. Smith reported that Dr Wittmack, Dr Magnus, and Dr Brefeld of Berlin had examined his preparations and considered that the compound spores belonged to *Urocystis*. He adds, 'Dr Brefeld says he has seen the Urocystis growing upon very different materials, even upon bread'. In my opinion there is no doubt that Smith's fungus was a species of *Papulaspora*. The 'spore-balls' as figured by Smith are 42–45 μ diam. (which approximates to the average diameter of those of *P. dodgei*); each is composed of five to six central cells surrounded by a cortex of transparent cells which, like the central cells, 'burst, and throw out threads of mycelium', all features more characteristic of *Papulaspora* than of *Urocystis*.

Uredo gladioli Requien

U. gladioli Requien was first described by Duby (*Botanicon Gallicum*, II, p. 901, 1830) as '*U*[*redo*] *Gladioli* (Requien in herb. DC.) bifrons, maculis circa lutiscentibus, acervulis nigris suborbicularibus sparsis confertisque convexis epidermide bullatâ clausâ tectis, sporidiis subglobosis sessilibus reticulo pellucido tenuissimo obvolutis.—In Gladiolo circà Avenionem (cl. Req.).'

Again no type specimen has been located with certainty, but in the De Candolle Herbarium of the Delessert Herbarium, Geneva, there are two gladiolus leaves in the *Puccinia gladioli* Cast. folder which were collected by Requien, also at Avignon, in 1819. Through the courtesy of the Director of the Conservatoire et Jardin Botanique, Geneva, it has been possible to examine this specimen which, as the presence of teliospores proves, has been correctly classified. The original label attached to the specimen bears the words (in handwriting which may be Requien's) 'Xyloma? gladioli nob. Avignon' with the addition by De Candolle of 'Mr Requien 1819'. This specimen must have been diagnosed as *P. gladioli* subsequent to the description of the gladiolus rust by Castagne in 1842. It may well be the one on which Duby based *Uredo gladioli* Requien, especially as Linder (in Hotson, 1942*b*) records that there is a specimen of the telial state of the gladiolus rust in the Curtis Herbarium (Farlow Herb.) labelled by Curtis '*Uredo gladioli* Duby!' The first part of Duby's description suggests the rust, but the last four words are similar to those applied to *U. ranunculacearum* [*Urocystis anemones*] described on the same page. In the face of the above evidence the safest course is probably to reject *Uredo gladioli* Requien as a *nomen ambiguum* under Art. 62 of the International Rules of Botanical Nomenclature on the grounds that De Toni applied the name to a smut; Dodge and Laskaris (1941) used it for a species of *Papulaspora*, while Sydow (1904) listed *Uredo gladioli* Req. as a synonym of *Puccinia gladioli* Cast. The gladiolus smut is, therefore, now proposed as a new species.

Urocystis gladiolicola n.sp.

Sori in the leaves, as dark brown blisters parallel with the veins 1 mm. to several cm. in length, at first covered by the epidermis which ruptures to expose the spores, and in the corms. *Spore mass* powdery, dark brown. *Spore balls* globose, 15–28 μ diam., each composed of one or two spores completely surrounded by a rather irregular layer of colourless sterile cells, 6–10 μ diam. *Spores* globose or slightly angled, reddish brown, 12–17 μ diam.

On cultivated gladiolus, Stoke Climsland, Cornwall, England. Collected by A. Beaumont, June 1944. (Herbarium Plant Pathology Laboratory, Harpenden, Herts, No. 14, Type.)

Sori foliicoli vel in cormis efformati, in foliis pustulas atro-brunneas cum nervis parallelis, 1 mm. ad plures cm. longas efficientes, primo epidermide tecti, dein erumpentes. *Massa sporarum* atro-brunnea, pulverulenta. *Glomeruli* globosi, 15–28 μ diam., e 1–2 sporis centralibus, cellulis sterilibus hyalinis, irregulariter dispositis 6–10 μ diam. circumdatis constantes. *Sporae* globosae vel leviter angulatae, rufo-brunneae, 12–17 μ diam.

Hab. in foliis cormisque Gladioli, Stoke Climsland, Cornwall, Anglia; leg. A. Beaumont, Jun. 1944. (Herb. Path. Lab., Harpenden, Herts, No. 14, Typus.)

Fragments of the type specimen (which has been figured by Moore (1948), figs. 25, 26) have been deposited in the Herbarium of the Royal Botanic Gardens, Kew and the Herbarium of the New York Botanic Garden.

Attempts made in November 1947 to germinate spores of the type specimen were unsuccessful.

I am indebted to the Keeper of the Herbarium of the Royal Botanic Gardens, Kew, and to the Keeper of Botany of the British Museum (Natural History) for allowing me access to the collections in their charge, to Mr W. C. Moore for his kindness in supplying specimens, and to Miss E. M. Wakefield for improving my Latin.

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NEW RECORDS AND OBSERVATIONS. IV

By A. A. PEARSON, F.L.S.

(With Plate XXII and 3 Text-figures)

This paper is divided into two sections. The first deals with species not previously recorded for Britain, but which were included in the Revised List of British Agarics and Boleti by Pearson and Dennis, published in these *Transactions*, vol. XXXI. The rest of the paper covers additions to the list and notes on uncommon or little-known species.

Section 1

Lepiota serena (Fr.) sensu Kühner. *Bull. S.M. Fr.* LII, 213.

Pileus 1–4 cm. fleshy, conico-campanulate with or without umbo, white, covered with silky radial fibrils, smooth or rimose-squamulose; margin smooth or appendiculate with veil. *Gills* crowded, white or creamy white, linear or ventricose, free, edge fimbriate. *Stem* 1–6 cm. long either equal or with bulbous base, sometimes strikingly ventricose, white, solid or narrowly fistulose. *Ring* superior, membranous, white. *Flesh* white or grey. *Taste* mild but rather rancid. *Smell* strong, pleasant. *Spores* white, elliptic-oblong, $7–8 \times 3\frac{1}{2}–4\frac{1}{2}\,\mu$, 1 guttulate, smooth without germinating pore, turn red in iodine. *Cystidia* on gill edge clavate or mixed with cylindrical cells, $12–30\,\mu$ wide.

Habitat: on ground or among dead leaves. N. Devon, September 1945 and October 1946. Wiltshire early November 1947. Very variable in size but easily recognized by the guttulate spores and rather voluminous cystidia.

There are several interpretations of *L. serena*. It was referred by Boudier to Cooke's Ill. 47 (57) of *Armillaria subcava* which is more likely to be *Lepiota brebissonii* Godey. The *L. serena* of Lange in *Fl. Ag. Dan.* I, 29, Pl. 11B has cystidia with a long beak and has been referred to *L. sericea* Cool by Huysman in his *Observations sur le Genre* '*Lepiota*': *Med. Ned. Myc. Ver.* XXVIII.

L. serena seems to be fairly common in the west of England and has been sent to me on several occasions by F. R. Elliston Wright, Braunton, N. Devon, and T. W. Dunston, Donhead, Wiltshire.

Lactarius representaneus Britz. *Hym. a. Sudb.* (1885).

Pileus up to 16 cm., convex or flat and depressed, sometimes with small umbo, viscid, bright straw colour, violet when rubbed, glabrous in centre, elsewhere with adpressed scales; margin incurved and shaggy. *Gills* pale cream, thin, crowded, adnate or subdecurrent staining violet. *Milk* pale cloudy cream, unchangeable when isolated on a glass slide, but turning the flesh violet; taste like wild thyme then slightly bitter. *Stem* up to 10 cm. long, 25 mm. diam., hollow, subequal or slightly ventricose, somewhat viscid or weeping when moist except at pruinose apex, then dry and glabrous, straw or golden yellow, pitted with shallow dull yellow hollows. *Flesh* pale cream, turning violet. *Taste* like the milk. *Smell* faint. *Spores* subspherical cristate echinulate 9–11 × 7½–9 μ. *Cystidia* fusoid acute 60–70 × 10–12 μ.

Habitat: in moist boggy places among leaves and moss. Found in Rothiemurchus Forest, Scotland.

This robust species is allied to *L. scrobiculatus* from which it differs in the flesh turning violet instead of yellow, also by the taste and brighter colour.

An excellent description and a beautiful coloured plate by Jules and Jeanne Favre are given in *Bull. Soc. Myc. Fr.* LV, Atlas, Pl. LXXXI (1939).

Russula rosea Quél.

In these *Transactions*, XXII, 36 and XXIII, 307, *R. aurora* Krombh. and *R. rosea* Quél. were described separately, but the two descriptions should be combined and the least ambiguous of the two names would appear to be *R. rosea*. The range of colour is from a delicate pink to that of a ripe peach. The latter colouring is uncommon, while the pink form is fairly common and often recorded as *R. lepida*. The chemical reaction to sulphovanillin which turns the flesh of *R. rosea* to a brilliant carmine red is a useful and decisive diagnostic feature.

Many authors have used the name *R. aurora* for this species and it would have priority if it were certain that it is the same, but its identity is doubtful. Krombholz describes it as having a reddish stem and this suggests a form of *R. lepida*. In his Appendix II, *Trans. Brit. mycol. Soc.* XVII, 44, Carleton Rea has already recorded *aurora* Krombh. as a variety of *lepida* on the basis of Bresadola's description and figure, Tab. 414, in *Icon. Myc.* and this probably is a form of *R. lepida* but quite distinct from *R. rosea*. It is proposed for the present to adopt *Russula rosea* Quél. and record the peach-coloured form as forma *aurora* Pearson.

Entoloma turbidum Fr.

Pileus 2–4 cm., fleshy, campanulate then expanded with obtuse or more rarely with acute umbo and undulate margin, radiately splitting when old, smooth, polished; almost black at first, then brownish or drab-grey (near Buffy Brown: Ridgway), darker in centre; margin inturned at first, cuticle slightly overlapping when expanded. *Gills* subdistant, grey, then pinkish grey, ventricose, broad, veined, adnate-emarginate then free, edge wavy. *Stem* 5–10 cm. long, thick set, sometimes even, but in typical forms clavate with very swollen base, often connate, rather firm, white with blackish grey striate fibrils, base white tomentose, stuffed with loose fibres. *Flesh* white. *Taste* mild. *Smell* faint or none. *Spores* in mass Vinaceous Cinnamon (Ridgway), subglobose apiculate with irregular outline but no distinct angles, contents granular-vacuolar 8–9 × 6½–7 μ including apiculus. *Cystidia* absent. *Epicutis* of cap: septate hyphae 7–9 μ wide with greyish brown granular contents and hyaline clamp connexions.

Habitat: in mixed wood including birch. Brockenhurst, New Forest, Hampshire, 20 October 1947; also in Churt, Surrey, 14 October 1940.

The above corresponds to the type as described by Fries in *Monographia*,

p. 276, but as he there mentions, *E. turbidum* is found *sub plurimis formis*, many of which are not so clearly characterized as the New Forest specimens. The species figured by Lange in *Fl. Ag. Dan.* II, p. 96, Pl. 76 H is quite different and suggests a large *Nolanea*.

Naucoria arvalis Fr. and var. **tuberigena** Quél. 17 *Suppl. Ch. Jura* and *Vosges* (1889) as *Galera arvalis* var. *tuberigena* = *Naucoria sclerotina* Velenovsky in *Mykologia*, Prague, 1924, p. 144.

Pileus 1–2 cm., convex then flat, gibbous or with shallow umbo, somewhat fleshy in centre, membranaceous elsewhere, hygrophanous, dull ochre or honey colour or with a reddish tinge when moist, then bright ochraceous yellow, smooth, often rugulose with fine radiating veins, oily looking when moist, mat when dry, margin not striate with cuticle slightly overlapping gills. *Gills* crowded, pallid then brown adnate, more or less emarginate, linear, ventricose or wedge-shaped, edge straight or slightly wavy and fimbriate. *Stem* long and slender, 6–12 cm. × 2–4 mm. thick, white or pallid buff, darker below, tough and elastic with internal pith, equal or swollen below either with an attenuated rooting base or abruptly attached to a rugulose brownish black soft sclerotium 1–4 cm. *Flesh* honey colour in cap, paler in stem. *Taste* bitter. *Smell* none. *Spores* in mass snuff brown, elliptical with truncate apical pore $9–10 \times 5–5\frac{1}{4}\,\mu$., smooth. *Cystidia* on gill edge flask-shaped with pointed or blunt apex about $40 \times 12\,\mu$; on gill face club-shaped with 2 to 5 sterigmoid filiform appendages—mostly 3 in the specimens examined. Upper surface of cap hymeniform with sack-shaped cells mixed with fusoid cystidia.

Habitat: among débris of various kinds. Hindhead, Surrey, 30 September 1944, in garden. Near Haslemere, 9 September 1945, on rotting straw heap. Grayshott, Hampshire, among loose roadside sweepings.

The sclerotium was not seen in the first two gatherings, but in the last there were two fruit-bodies attached to a sclerotium the size of a walnut. This is much larger than the sclerotia hitherto recorded.

N. arvalis is probably fairly common and may be passed as a form of *N. semi-orbicularis* from which it differs in many particulars, notably in the bitter taste and remarkable cystidia on the gill face with their finger-like projections; also by the sclerotium which is not always present but may be overlooked and left in the ground.

There is a plate in Cooke's *Illustrations*, 506 (479), from a drawing by Worthington G. Smith which depicts specimens growing on sand at Yarmouth. Cooke labelled it *Agaricus* (*Naucoria*) *arvalis* Smith (not Fries). It probably is the same species. Agarics growing on the seashore often 'suffer a sea change'.

Inocybe squamata Lange. Jakob E. Lange, *Dan. Bot. Ark.* Bd. 2, Nr. 7 (1917) and *Fl. Ag. Dan.* III, p. 83, Pl. 114A (1938).

Pileus 2–10 cm., convex, umbonate then flat and finally upturned with umbo obsolete or persistent, tomentose-arachnoid, olive buff with rusty patches, darker in centre (sec. Lange breaks up into scales on disk). *Gills* crowded, at first dingy yellow (Old Gold: Ridgway) then olivaceous brown with white fimbriate edge, adnate emarginate or almost free, ventricose. *Stem* 3–7 cm. long, 5–10 mm. thick, stuffed with loose pith then hollow, olive-buff then reddish ochre, striately fibrillose with shaggy scales, equal or sub-bulbose, white tomentose at base. *Flesh* olive to ochraceous buff, ferruginous in pith of stem and with horny line above the gills. *Taste* mild. *Smell* none. *Spore powder* snuff brown. *Spores* elliptical subphaseoliform smooth 8½–9½ × 5–5½ μ. *Cystidia* on gill edge densely packed, cylindrical or sack-shaped with thin walls. *Epicutis* of cap with hyphae 6–10 μ septate with small clamp connexions.

Habitat: gregarious; in moist land under oak, birch and willow, 2 October 1946.

The above description was taken from a large troop with caps ranging in size from 2 to 6 cm. wide, some with robust stems 7–10 mm. diam. They differ in the diagnosis of Lange by the absence of scales in the centre of the cap, but Lange may have seen old specimens only.

I. squamata must be rare as it is such a very striking fungus that otherwise it could hardly have been overlooked. It belongs to the group without thick-walled cristate cystidia and is close to *I. fastigiata* from which it differs totally in colour and has much smaller spores.

Inocybe descissa Fr. var. **brunneo-atra** Heim in *Le Genre Inocybe*, p. 234 (1931).

Differs from the type in the cap being radially streaked with dark brown almost black fibrils; often split showing white flesh underneath. *Cystidia* ventricose-fusoid with long neck and cristate apex 50–80 × 18–25 μ. *Spore powder* snuff brown. *Spores* subamygdaliform, smooth, 9–10 × 5–6 μ. Not uncommon.

Another fairly common variety is what Lange has described as *I. microspora* the spores being 6½–7 × 4½–5 μ, but this is close enough to the original Friesian diagnosis of *I. descissa* to be considered the type, so for the present it may be left in the British list as such.

The following notes should be added to the 'Revised List':

Pleurotus applicatus (Batsch) Fr. has smooth globose spores 4–6 μ. We have gathered it recently.

Pleurotus applicatus sensu Quélet, Rea non alt. = *P. silvanus* Sacc. This is the species with smooth elliptical bean-shaped spores 7–8 × 3–4 μ.

Crepidotus applanatus (Pers.) Fr. has round warted spores 5–6 μ. It has been retained in the list but requires confirmation.

Crepidotus applanatus sensu Quélet, Rea non alt. = *C. fragilis* Joss. (1939) = *C. autochtonus* Lange (1938). This is the species with elliptical or rather almond-shaped spores 7–9 × 5–5¾ μ. The valid name is *C. fragilis*.

We owe the clearer elucidation of the above four species to M. Josserand in *Bull. Soc. Myc. Fr.* XLIX, 360–4 and LIII, 218.

SECTION 2

Mycena olida Bres. *Fungi Trid.* I, 73 (1887) and *Icon. Mon. Tab.* 240 (1928) = *M. trachyspora* Rea, *Trans. Brit. mycol. Soc.* XII, 216 (1927) = *M. gypsea* Lange non Fr. *Dansk. Bot. Ark.* Bd. 1, Nr. 5, 26 (1914) and *Fl. Ag. Dan.* II, 41, Pl. 52B (1936).

Pileus 5–20 mm., thin, conical, obtuse, rarely with acute umbo, sometimes striate by transparence, but usually opaque; white with slight yellow tint especially in centre; glabrous or delicately and innately fibrillose. *Gills* crowded, white, adnate-sinuate then free, linear or ventricose. *Stem* 1–3 cm. long, 1–2 mm. diam., white, smooth to the eye but minutely pubescent *sub lente*, equal or slightly attenuated above, tomentose at base. *Flesh* white. *Taste* mild. *Smell* none or faint of new meal when pressed between fingers. Gill edge more or less fertile with scattered projecting *cystidia* which are cylindrical or flask-shaped mostly with blunt apex 40–60 × 6–10 μ. *Basidia* 2 or 4 spored. *Spores* in mass white, non-amyloid, ovate apiculate, smooth with granular contents or 1-guttulate, mostly about 6½ × 4½ μ in British collections which had basidia with 4 sterigmata, but the full range is 6½–9 × 4½–5½ μ sec. Kühner.

Habitat: caespitose on the dead trunks or stumps of deciduous trees. Lessness Abbey Woods near Woolwich, November 1926, and 1927, on old elm stump, Churt; on beech stump 15 November 1947.

It is tempting to identify this species with the long-lost *M. luteo-alba* Bolton which, however, Fries associated with pine woods. *M. olida* may be more common than we have thought and has been overlooked because it appears on stumps in a small form very like *M. flavo-alba*. The larger form which Rea named *M. trachyspora*, through an incorrect observation of the spore, is rare in the south of England. It is said to be common in France where it occurs chiefly on horse chestnut, also on ash, beech, elm, poplar and sycamore.

Lange recorded this as a form of *M. gypsea*, a species which is closely related to *M. lactea* (Pers.) Fr. but with more definitely fusiform spores and less conical cap, which I have found growing in troops in a grassy avenue often attached to fragments of *Cupressus* twigs.

Mycena clavicularis Fr.

Pileus 5–20 mm., dry, membranaceous, subglobate, campanulate or subconical, often with central papilla which flattens or collapses into a small central umbel; dingy white with black or brown striae reaching to the brownish disk. *Gills* crowded, smoky grey or greyish white with white edge, rather broad, ventricose or wedge-shaped, adnate with decurrent tooth. *Stem* 4–5 cm. long, 1–1½ mm. diam., hyaline or with smoky tint, smooth, polished, cartilaginous-elastic, very viscid. *Flesh* blackish in cap, white in stem. *Taste* mild. *Smell* none or faint. *Spores* in mass white, amyloid, oblong elliptic or obovate, apiculate, 7–8 × 5–5½ μ. *Cystidia* on gill edge densely packed with pyriform brush-like cells, 10–15 μ, set with warts.

Habitat: in troops under conifers; North Heath near Midhurst, Sussex, 23 October 1947.

This species is replaced in the British list and may be fairly common in coniferous woods after a downpour of rain. The stem is very sticky, but only the stem; there is no separable glutinous cuticle on the cap or filament on the gill edge as in *M. epipterygia* and other allied species of this group.

In these *Transactions*, XXII, p. 30, was described under the binomial *Collybia pseudo-clusilis* Joss. and Konr. a small agaric which at first I wanted to call *C. cessans* Karst., but deferring to the opinion of my old friend

Carleton Rea, it was identified with the first-named. Since then I have had occasion to reconsider this matter and think it desirable to withdraw *C. pseudo-clusilis* and replace it by *C. cessans* Karst. sensu Lange. The two species have some points in common, but as Josserand (*in litt.*) has pointed out, *C. pseudo-clusilis* is of a gelatinous nature and quite distinct from our species. The points at issue have been further involved by the collection of another agaric of a remarkable gelatinous character, also very reminiscent of *C. pseudo-clusilis*, but with amyloid spores, which like the other two species occurs in the late autumn and winter. It has been suggested by both Kühner and Josserand (*in litt.*) that this may be a grey form of *Omphalia maura*. This may be so, but it looks so very distinct from the type that further observations are called for. The usual habitat of *O. maura* is on burnt ground. It is possible that when it occurs elsewhere the whole aspect of the fungus may change.

Collybia cessans Karst. (1871 in *F. et Fl. Fenn. Notis.* XI, p. 219) sensu Lange in *Dansk. Bot. Ark.* Bd. 2, Nr. 3, p. 21 (1917) and *Fl. Ag. Dan.* II, 17, Pl. 46A (1936).

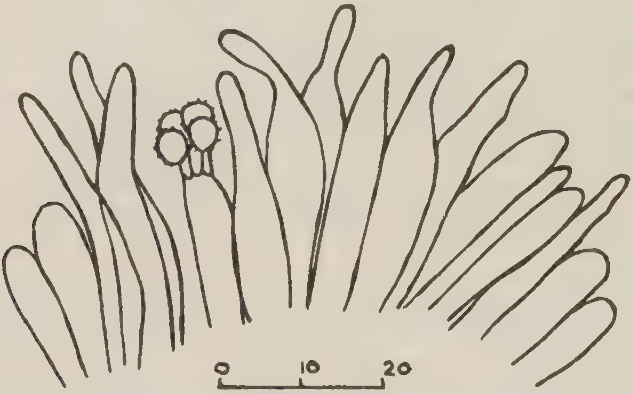
Pileus 1–2 cm., convex then flat, depressed or umbilicate, pale grey with darker striae by transparence, soon opaque and when dry, silky silvery grey, polished, smooth. *Gills* distant, white or dingy white, ventricose, rather broad, emarginate with decurrent tooth, fairly thick with blunt edge which is smooth or minutely pubescent. *Stem* 1½–2 cm. long, 1–1½ mm. thick, grey, paler at apex, equal or slightly thicker above, polished. *Flesh* thin, pale grey drying to white. *Taste* mild. *Smell* none. *Spores* in mass white, non-amyloid, broadly oval to subglobose, apiculate, 5½–7 × 4½–5 μ, with granular contents or 1-guttulate. *Cystidia* on gill edge fringed with abundant projecting cells, cylindrical or slightly swollen below, 40–60 × 8–10 μ. *Epicutis* of cap with septate hyphae 6–10 μ diam.

Habitat: on ground, usually in grass under conifers; solitary or in troops. Fairly common in November and December.

Pleurotus palmatus (Bull.) Fr. = *Rhodotus palmatus* (Bull. ex Fr.) Maire.

During the fungus foray of the Yorkshire Naturalists Union on 13 October 1947, several large specimens of this species were found growing on a stump near Jervaulx Abbey. The whole surface of the cap was ornamented with a network of raised veins. The opportunity was taken to verify some points. It has been stated that the spore powder differs in colour when a deposit is taken without the gills touching paper. Our specimens gave abundant deposits and both with and without contact of the paper the colour of the spores in mass was Pinkish Buff (Ridgway). This conflicts with the observations of René Maire and A. Pouchet, both of whom have stated that the colour is rusty brown when the deposit is made in contact with the paper. This may well happen when the specimens are so damp that the dirty moisture colours the spores, a not uncommon effect with any moist agaric when part of a spore print may be one colour and part another.

Pouchet, in *Bull. Soc. Myc. Fr.* XLVIII, 76–83, has given figures of the cystidia on the gill edge. These are not easy to observe. A microtome section of the gill was prepared and confirmed Pouchet's observations. The edge is occupied by a narrow belt of very thin-walled fragile cystidia with densely staining protoplasm contents occupying even the tip of the 'beak'. The accompanying *camera lucida* drawing, kindly supplied by Dr R. W. G. Dennis, illustrates this.



Text-fig. 1. Edge of gill of *Pleurotus palmatus*

Russula smaragdina Quél. Suppl. 14, t. 12 (1885).

Pileus 2–3 cm., convex, pale lemon yellow, mat, margin smooth without striae. *Gills* subcrowded, ventricose adnexed, white, edge smooth. *Stem* 3 cm. tall, 5 mm. diam., white, solid, equal. *Flesh* white. *Taste* mild (or with a faint acrid after-taste sec. Lange). *Spores* white in mass, globose or subglobose, $6\frac{1}{2}$–$7\frac{1}{2} \times 6\frac{1}{2}$–$7\,\mu$, reticulate all over, with no secondary finer network when seen under high magnification. Outline of spores with sixteen to twenty shallow projections. *Cystidia* on gill edge crowded, fish-shaped, mostly with blunt apex, turning dark blue all over with sulphovanillin; on gill face sparse; on cuticle of pileus numerous.

Habitat: oak woods, Derrycunnily Falls, Killarney, 3 September 1946. Rare.

Some authors have identified this small species with *Russula aeruginea* Lindbl., but in many of its characters it is quite distinct.

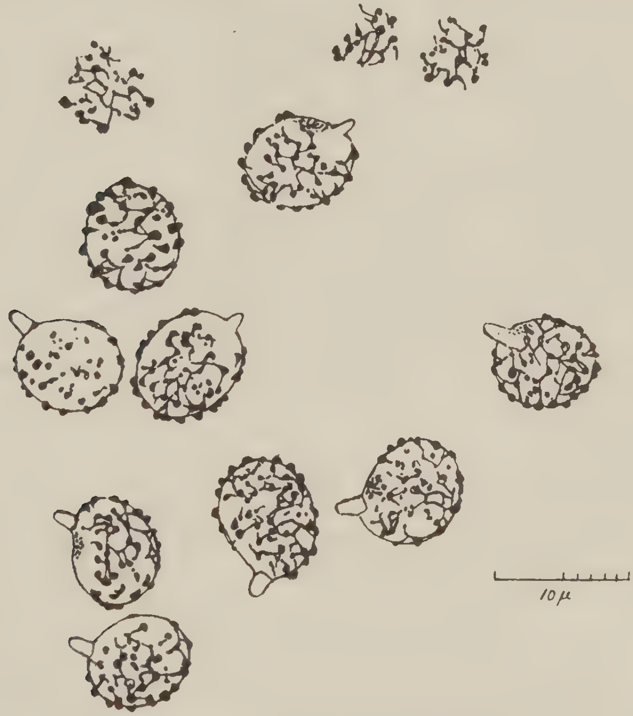
Russula curtipes Möller & Schaeffer. *Bull. Soc. Myc. Fr.* LI, 108–12.

Pileus 5–12 cm., convex depressed, creamy buff or mottled with brownish red, deepening in colour towards margin which is reddish brown or purplish red, at first with a white tomentose or flocculose surface especially on disk, then smooth and mat, sometimes cracking; cuticle peels at margin which is only slightly striate-tuberculate when old. *Gills* crowded or subdistant, cream at first then the deeper ochraceous colour of the spores, spotted brown in parts, rounded in front, attenuated towards stem and minutely emarginate, anastomosing at base and often forked near stem. *Stem* 3–7 cm. long, 2–4 cm. diam., usually dumpy, white turning foxy brown when handled, often thicker above or below, pruinose or flocculose at apex, smooth or rather silky below, rugulose, solid and hard. *Flesh* white, compact. *Taste* mild. *Smell* pleasant. *Chemical reactions* iron sulphate, flesh pink or greyish. *Spores in mass* ochraceous F of Crawshay's chart. *Spores* broadly oval with prominent apiculus, blunt warts and short or sometimes rather long crested ridges 7½–10 × 6½–8 μ. *Cystidia* on gill edge not very abundant, obtuse or lanceolate, stain bluish black in sulphoranillin. *Epicutis* of cap with narrow vermiform hyphae only faintly staining blue in sulphoranillin, 4–5 μ wide.

Habitat: under beeches. Cowdray Park, Midhurst, Sussex, 3 and 22 August 1946.

This robust species may prove to be not uncommon. It has doubtless been confused with *R. alutacea* or *R. romellii* or even *R. xerampelina* from which it can be distinguished by the cristate spores.

Mr F. H. Møller, one of the authors of this species, kindly confirmed my determination of the specimens gathered at Cowdray Park.



Text-fig. 2. *Russula scotica* Pearson, basidiospores.

Russula scotica Pearson.

In these *Transactions*, XXIII, 310, Pl. IX (1939), a new species of *Russula* was described and illustrated. The spores were drawn by me somewhat diagrammatically. Thanks to the kindness of Mr Richard Crawshay, I am able to give his *camera lucida* drawings of the spores of *R. scotica* which show their ornamentation much more accurately. These drawings will appear in the second edition of Mr Crawshay's book, *Spore ornamentation of the 'Russulas'*.

Highly magnified and in an iodine solution which stains only the armature of the spores, they are seen to have prominent warts and a rather coarse asymmetrical network, due to lines connecting the warts. The average size is 8½ × 7¼ μ.

Entoloma cordae Karsten = *Rhodophyllus cordae* (Karst.) Lange in *Fl. Ag. Dan.* II, 97, Pl. 76D.

Pileus 1½–3 cm., rather fleshy, convex or flat with shallow umbo, grey with slight brown tinge drying to grey with black centre, silky, polished, not radiately fibrillose but minutely pubescent, striate when fresh, striate by transparence when fresh, then opaque. *Gills* distant, white then grey and finally with pinkish tinge, broad, ventricose, veined, adnate-sinuate then free. *Stem* usually tall, 5–6 cm. long, 4–6 mm. thick, white, striate, silky, equal or thicker below, often with pointed base, flexuose, fragile, hollow. *Flesh* dark grey in cap drying to dingy white and white in stem. *Taste* mild. *Smell* none or faint. *Spores* in mass Vinaceous Cinnamon (Ridgway), subglobose with prominent apiculus, outline only slightly irregular with granular vacuolar contents 8–10 × 6½–7 μ including apiculus. Some of the spores are more definitely angular than others. *Cystidia* absent. *Cuticle* of cap with pale yellowish brown septate hyphae very indistinctly defined under the microscope.

Habitat: on ground under conifers; from Braunton, N. Devon, 25 October 1947. Under larch sent by Dr F. R. Elliston Wright; in pine wood, Great Common near Midhurst, Sussex, 6 November 1947.

This species belongs to what Romagnesi (*in litt.*) calls the '*turbidum*' group which have small roundish subangular spores with vacuolar contents. It is, however, well-defined and worthy of specific distinction. From *E. sericeum* with which it can easily be confused, it differs in the absence of a mealy smell; the spores in the latter species are much more definitely angular.

Naucoria muricata (Fr.) Romagn. = *Pholiota muricata* Fr.

Pileus ½–2 cm., rather fleshy, semi-globate, reddish brown, pulverulent with fugacious warts. *Gills* crowded, rather broad, clay colour then brown with white fimbriate edge, adnate, ventricose. *Stem* slender, 1½–2½ cm. long, 1½–3 mm. thick, covered with a fugacious mealy coating like the cap, reddish brown, paler and pruinose at apex. *Flesh* buff. *Taste* ? *Smell* pleasant. *Spore powder* brown. *Spores* pale yellow under microscope, bean-shaped, smooth, 7–8 × 4–4½ μ. *Cystidia* on gill edge with a fringe of flexuose hairs 5–6 μ diam. with swollen tips 9–10 μ wide. *Epicutis* of cap with globose or pyriform cells 18–20 μ, surface slightly rough.

Habitat: in troops on old sawdust and chips of wood. Ross Island, Killarney, Eire, 1 September 1946. The normal habit is on fallen branches of deciduous trees.

The above looks very like *Naucoria siparia* Fr. which differs in the cap being covered with erect hairs built up from brown septate hyphae with a vaguely rough surface and clamp connexions. *N. siparia* I have gathered in Kent and it has also been sent from N. Devon. There are at least two other species with which it could be confused, notably *N. granulosa* Lange which has not yet been recorded in this country, and *N. erinaceus* Fr. (=*Pholiota erinacea* (Fr.) Quél.) which turns up from time to time but has a much more scaly surface.

N. muricata is now replaced in the British List, but transferred to *Naucoria* in order to link it up with nearly related species.

Naucoria scolecina Fr. sensu Lange in *Dansk. Bot. Ark.* Bd. 6, p. 20 (1938) and *Fl. Ag. Dan.* IV, 21, Pl. 125H (1939) = *Alnicola badia* Kühner in *Bull. Soc. Myc. Fr.* XLVII, 239 (1931) = *Tubaria umbrina* R. Maire in *Bull. Soc. Myc. Fr.* XLIV, 48 (1928).

Pileus 1½–2½ cm., flat or with shallow umbo, hygrophanous, rusty bay or umber, smooth or with a furfuraceous pruinose surface, opaque or striate half way to centre. *Gills* subdistant, ventricose or arcuate, adnate or subdecurrent, concolorous, edge pallid. *Stem* slender, blackish or reddish brown, paler at apex, often white at base. *Flesh* dark brown when moist. *Smell* none or slightly raphanoid. *Taste* mild. *Spores* brown, almond or boat-shaped, minutely verrucose, 9–11 × 5–5½ μ. *Cystidia* on gill edge fringed with lanceolate cells having a bulbous base and long slender acute beak.

Habitat: in Alder swamps. Collected in Surrey and Sussex on several occasions.

The microscopic features of this species are practically the same as those of the common *Naucoria escharoides* which when fresh is a pale *café au lait*, but when old can easily be taken for the above. Lange has confused matters by stating that *N. escharoides* has elliptical spores, whereas they are pointed at both ends. The two species differ in colour, gill spacing and taste.

Neither of the epithets applied to the above species by Maire and Kühner are valid, both having been used elsewhere, but in a recent paper by Kühner (*Ann. Univ. Lyon*, 1942, p. 7) it is suggested that as another name has to be found, the one adopted by Lange should be accepted though what the original *Naucoria scolecina* was, none can say. Modern definitions of small agarics are largely based on microscopic observations which were not available in the days of Elias Fries.

Galera clavata (Velenovsky) Kühner in *Le Genre Galera*, p. 171 (1935) as *Galerina clavata*. Velenovsky in *Ceské houby*, p. 548, as variety of *Galera fragilis* Vel. non Peck (1921).

Pileus 1–3 cm., thin, obtusely conical or semi-globate, ochraceous, hygrophanous drying to buff, smooth, striate at margin or to disk.

Gills distant, broad, pale ochre or honey colour at first, ventricose, adnate-emarginate. *Stem* 3–7 cm. long, ¾–2 mm. thick, honey or ochraceous, equal, sometimes flexuose, pruinose above. *Flesh* pale ochre. *Taste* mild. *Smell* none. *Spores* in mass, rusty ochre (Antique Brown: Ridgway), elongate elliptical or obovate, with lateral apiculus 12–14 × 6–7 (11–16½ × 6–8, 7 μ Kühner). *Basidia* with 2 or 4 sterigmata. *Cystidia* on gill edge skittle-shaped with long neck and globose apex, abundant or sparse. *Epicutis* of cap made up of septate hyphae 7–16 μ.

Habitat: in large troops on lawn, Cowdray Park, Sussex.

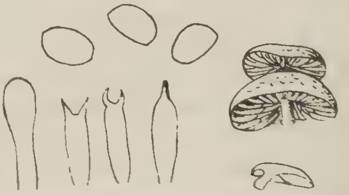
This species is characterized by the large spores and long-necked skittle-shaped cystidia.

Galera nana (Petri) Kühner.

A description of this species was given in these *Transactions*, XXVI, 48, but the spore measurements were omitted. They are almond-shaped minutely verrucose 10–11 × 5–6 μ.

Crepidotus hibernianus sp.nov.

Pileus 5–15 mm., lateral, membranaceous, oblong shell-shaped, fibrillose-rimose, covered with small ferruginous scales; margin incurved and with traces of a white veil giving a denticulate appearance to the edge. *Gills* subdistant, narrow, linear, pallid buff then ochraceous or dark ferruginous, edge strikingly serrate-fimbriate. *Stem* rudimentary, obconical, ochraceous, pubescent. *Taste* mild or slightly bitter. *Smell* faint or none. *Flesh* light yellow. *Basidia* with 2 sterigmata, 15–20 × 4–5 μ, oily contents reddish brown. *Spores* brown, broadly oval, smooth, 8–10 × 6–7 μ. *Cystidia* on gill edge sterile with club-shaped cells 6–7 μ wide in tufts. *Epicutis* of cap formed of rather loosely woven thin-walled hyphae 4 μ thick, with reddish brown oily contents.



Text-fig. 3. *Crepidotus hibernianus* Pearson & Dennis. Basidia, spores and cystidia. ×650.

Habitat: on log of *Tilia europaea*. Muckross Park, Killarney, Eire, 29 August 1946.

Type specimens at the Herbarium, Royal Botanic Gardens, Kew.

This striking little species was gathered during a fungus foray at Killarney which Dr R. W. G. Dennis and I enjoyed in the genial company of several members of the Irish Naturalists Society.

LATIN DIAGNOSIS: **Crepidotus hibernianus** Pearson & Dennis. *Pileus* 5–15 *mm. lateralis, membranaceus, oblongulo-conchatus, fibrilloso-rimosus, minutis, squamulis ferruginosis; margine involuto; velo marginato blanco denticulato. Lamellae subdistantes, angustae, lineares, pallido-fulvae deinde ochraceae vel obscuro-ferruginosae; acie valde fimbriato-serrulata. Stipes incohatus, obconicus, ochraceus, pubescentis. Caro lutea pallida. Odor subnullus. Sapor dulcis vel leviter amarescens. Basidia clavata 2 sterigmatica* 15–20 × 4–5 μ. *Sporae fulvae, late ovales* 8–10 × 6–7 μ. *Acies lamellarum cystidiis clavatis* 6–7 μ *late, in fasciculis dispositis. Habitat: in trunco mortuo Tiliae europaeae. Killarney, Hibernia.*

Crepidotus pubescens Bres. *Icon. Myc.* Tab. p. 790 (1930) = *Dochmiopus terricola* (Britz.) Favre. *Schw. Zeitschrift für PilzKunde* XIII, 147 (1935).

Pileus 1–3 cm., thin, white turning ochre when dry or pale lemon from the first, reniform with two round lobes, minutely pubescent, attached to host on back of pileus; margin incurved. *Gills* crowded or subdistant of several lengths, rather wide, ventricose, edge blunt smooth or minutely fimbriate, free, radiating from an eccentric point, white, turning pinkish and finally brownish. *Stem* absent or reduced to an excentric white papilla. *Taste* rather rancid. *Smell* none. *Spore powder* clay colour. *Spores* pale yellow under microscope, smooth, elliptic with lateral apiculus 7–10 × 4–4½ μ or cylindric subfusiform 7–8 × 3–3½ μ. *Cystidia* on gill edge with cylindrical projecting cells, usually flexuose with blunt apex 6–7 μ wide. *Epicutis* of cap made up of septate hyphae 6–7 μ diam.

Habitat: on old leaves and stems especially bramble, nettle and bracken; very common.

This is the most frequent form in Britain of what hitherto we have called *Claudopus variabilis*. It appears, however, to be generally agreed that the specific epithet *variabilis* should be confined to the form with very minutely echinulate small spores about 6 × 3 μ. Bresadola's epithet has been adopted since Britzelmeyr's species is hardly well enough defined to call for its adoption.

It is a matter of opinion whether any of these forms deserve specific rank. The spore varies so much that on this character alone we could divide this little agaric into a long series based on spore shape and size.

The genus *Claudopus* should be confined to species with pink angular spores. French authors have adopted *Dochmiopus* Pat. for these small excentric agarics with what are usually described as pink spores. In a shallow spore deposit they do look pink, but when scraped into a small heap they are found to vary from light to dark clay colour or umber brown. There seems little justification for withdrawing them from *Crepidotus* in which group they were originally placed by Fries.

There is another type of angular or subangular spore which either has longitudinal ribs as in *Clitopilus prunulus*, or when the spore is looked at vertically, it is seen to have an irregular or polygonal outline with 7 or 8 sides, either obscurely angular or distinctly so. At least one species with this latter type of spore has the same field characters as *Crepidotus variabilis*; this is *Clitopilus pleurotelloides* (Kühner) Josserand, which has not yet been recorded for Britain.

Cortinarius (Hydrocybe) uraceus (?Fr.) sensu Lange. *Fl. Ag. Dan.* III, 45, Pl. 102 B.

Pileus 3–5 cm., conical with acute or obtuse umbo, at first sooty black then hoary brown with a very slight purplish tint, radially fibrillose, shining; margin incurved, then straight or upturned, often rather ragged and splitting, with scanty traces of a white veil when young. *Gills* subdistant, horny coloured or fuliginous in very moist specimens, finally rusty dark brown, ventricose or wedge-shaped, adnate, emarginate, rather thick. *Stem* 6–7 cm. high, 4–6 mm. thick, concolorous, striate with silvery fibrils, but the whole stem having a distinct purplish red tint; equal or swollen at base, stuffed with loose fibres, soon hollow. *Flesh* horn colour near surface of both cap and stem, whitish in centre. *Smell* strong of new meal. *Taste* rancid. *Spores* in mass chocolate brown (Argus Brown of Ridgway), broadly ovate with short lateral apiculus, finely echinulate 7–8 × 5–5¼ μ. *Cystidia* none.

Habitat: in troops under pine trees. Shiplake-on-Thames near Reading, 11 and 19 May 1947.

There are various interpretations of *C. uraceus*. The absence of an olive tint makes it doubtful if the above represents the species described by Fries, but for the present the name can be left unchanged.

Cortinarius (Hydrocybe) junghuhnii Fr.

Pileus 1½–2½ cm., conical with acute or obtuse umbo, fleshy in centre, buff colour (Warm Buff: Ridgway) at first minutely scaly from the white veil, the scales usually persistent but sometimes wearing off except round margin. *Gills* fairly crowded; arcuate, ventricose or triangular, 3–4 mm. broad, brown with white fimbriate edge sometimes strikingly serrulate, adnate, emarginate with or without decurrent tooth; often anastomosing at base. *Stem* 3–8 cm. long, 3–4 mm. thick, somewhat firm and cartilaginous, hollow, buff coloured-like cap, fibrillosely striate, equal or slightly swollen below, sometimes fusiform rooting. A vaguely defined white median ring may be present. *Flesh* buff. *Taste* mild. *Smell* none. *Spores in mass* reddish brown (Amber Brown: Ridgway). *Spores* pip-shaped with or without guttule, 7–8½ × 4¾–5½ μ, smooth or vaguely punctate. *Cystidia* on gill edge projecting in tufts, clavate of various lengths and diameters.

Habitat: under oaks near Gospel Green, Sussex, 25 September 1946.

Fries and Ricken give the habitat as in coniferous woods, Lange as under oaks, birches and pines. Lange also mentions the cystidia on gill edge. In our specimens all parts were buff coloured, but this may have been due to the dry weather prevailing at the time; normally the colour would probably be ochraceous cinnamon as given by Lange or pale brick colour as in the full description by Fries in *Monographia*, II, 113. The figures by Lange in *Fl. Ag. Dan.* Pl. 104E, only show the short-stemmed form, but among the large group gathered there were some specimens with a long fusoid stem as indicated by both Fries and Ricken.

Stropharia ferrii Bres. in *Riv. di Sc. Nat.* '*Natura*'. Milan, XIX, 17 (1928) and *Icon. Myc.* Tab. 842 (1931) = *Stropharia rugoso-annulata* Farlow (1929).

In these *Transactions*, XXIX, 201, Pl. 30, a description and figures of *S. rugoso-annulata* Farlow were given. In a recent number of the Swiss journal *Schw. Zeitschrift für PilzKunde*, January 1947, p. 12, A. Pilat has a note on a species found in Switzerland which he identifies with *S. ferrii* and synonymizes with *S. rugoso-annulata*. Another record from Switzerland was given by Métrod in *Bull. Soc. Myc. Fr.* LIII, 231–3 (1937).

The illustration by Bresadola gives an inadequate notion of the robust character of this species, but there seems little doubt that it is the same. It was first collected by Dr J. Ferri in northern Italy (Lombardy) and seems to be spreading. It is indeed strange that this very striking species first found in Italy, then in America, subsequently in Switzerland and England should have been overlooked until recent years.

Psilocybe catervata Massee.

Pileus 1–2½ cm., fleshy in centre only, campanulate-conical with obtuse umbo, white, when old with buff tint, minutely fibrillose *sub lente*, brittle. *Gills* subcrowded, umber brown with slight purplish tint, edge white fimbriate, linear or ventricose, adnate. *Stem* 3–4 cm. long, 2–2½ mm. thick, hollow but firm, white, striate with silky fibrils, equal or slightly swollen at base. *Flesh* white. *Taste* mild. *Smell* pleasant. *Spores* in mass sooty black, under microscope brown, elongate ellipsoid apiculate, truncate with germinating pore 10–12 × 5–6 μ. *Cystidia* on sterile gill edge ventricose with blunt or pointed apex 10–25 μ wide, mixed with sack-shaped cells. *Epicutis* of cap made up from short-celled septate hyphae 20–35 μ wide.

Habitat: on damp ground in troops; caespitose, near Jervaulx Abbey, Yorkshire, 13 October 1947.

Boletus leoninus Pers. *Myc. Eur.* (1825) = *Xerocomus leoninus* (Pers.) Quél.

Pileus 5–8 cm., convex then flat and depressed, dry, bright tawny or tan yellow with a slight sulphur or lemon tint here and there, changing to a pale reddish clay, covered with a close tomentum which is somewhat arachnoid, mat; margin incurved. *Tubes* and *Pores* wax yellow also with a very slight lemon tint, brownish when rubbed, adnate, emarginate, pores small, irregular. *Stem* lemon yellow above, dingy reddish brown below, ventricose, fusoid, minutely tomentose (*sub lente*), solid and firm. *Flesh* pure white almost unchangeable except for reddish tinge above the tubes and yellow near the surface. *Taste* mild. *Smell* strong, not unpleasant. *Spores* in mass Citrine (Ridgway), lighter in colour than the olive-green spores of many species; fusoid apiculate 10–13 × 5–5½ μ. *Cystidia* sparse, flask-shaped, hyaline.

Habitat: on ground of mixed deciduous woods including beech and birch; some pine trees not far away.

This was gathered by me at a Fungus Foray of the Bournemouth Natural History Society held at Brockenhurst, New Forest, Hampshire, on 20 October 1947. Two specimens were found from which the above description was made. *B. leoninus* has been recorded in the works of Micheli, Persoon, Krombholz, Quélet and Boudier, who in *Icones Mycologicae*, 1, Pl. 141 *bis*, has a coloured drawing all parts of which are tawny yellow except the flesh which is pale yellow—but his specimens were sent by post and we know what changes may take place in transit. He describes the cap as tomentose but cites Krombholz who states that the cap is completely naked. Persoon too says the cap is glabrous; Quélet that it is villous. In our specimens the cap (*sub lente*) was remarkably tomentose, but we cannot tell if this was partly due to the dry weather. A complete generalized description must wait till we know more about this very rare *Boletus*. I am indebted to E. J. Gilbert for help in identifying this species.

A rare *Hydnum*: During the Jubilee meeting of our Society a Fungus Foray took place at Windsor Park on 22 October 1946. In view of the cold weather prevailing at the time, little of interest was expected. However, several specimens of a creamy white *Hydnum* were brought in which perplexed all the experts present including our foreign visitors. These specimens were mostly without definite shape, with no pileus and only a few teeth in patches. Fortunately one good specimen was found with pilei clearly defined which I was able to take home. It proved to be *Hydnum diversidens*, a species which is not familiar to British mycologists. This may not be so rare as we have thought since it was brought in by several collectors, but in most cases it was growing in such inchoate shapeless masses that they would normally discard it as indeterminable. A description of the perfect example gathered is given below.

Hydnum diversidens Fr. = **Dryodon diversidens** (Fr.) Quél.

Pileus 9 cm. wide, 6 cm. thick, sessile, semicircular, flat or slightly depressed with obtuse but clearly marked margin, surface with blunt warts of irregular shape about 1 mm. long, cream colour (Cartridge Buff: Ridgway), pink when handled. Dried, the colour is tawny yellow streaked with orange. *Hymenium* concolorous with spines of various shapes, flat or round with blunt or toothed apices, mostly short, 1–2 mm., but longer ones present in the interstices. *Flesh* the same colour. *Taste* mild or slightly bitter after mastication. *Smell* not strong but pleasant. *Spores* white, subglobose or broadly elliptic, 1-guttulate, amyloid about $4 \times 3\,\mu$. *Gleocystidia* abundant, long and flexuose, 4–$6\,\mu$ wide, projecting like a fringe mostly with blunt apex, and with oily granular contents.

Habitat: on stumps in beech wood. Irregularly imbricate.

EXPLANATION OF PLATE XXII

Hydnum diversidens Fr. Specimen collected in Windsor Forest, 22 Oct. 1946.

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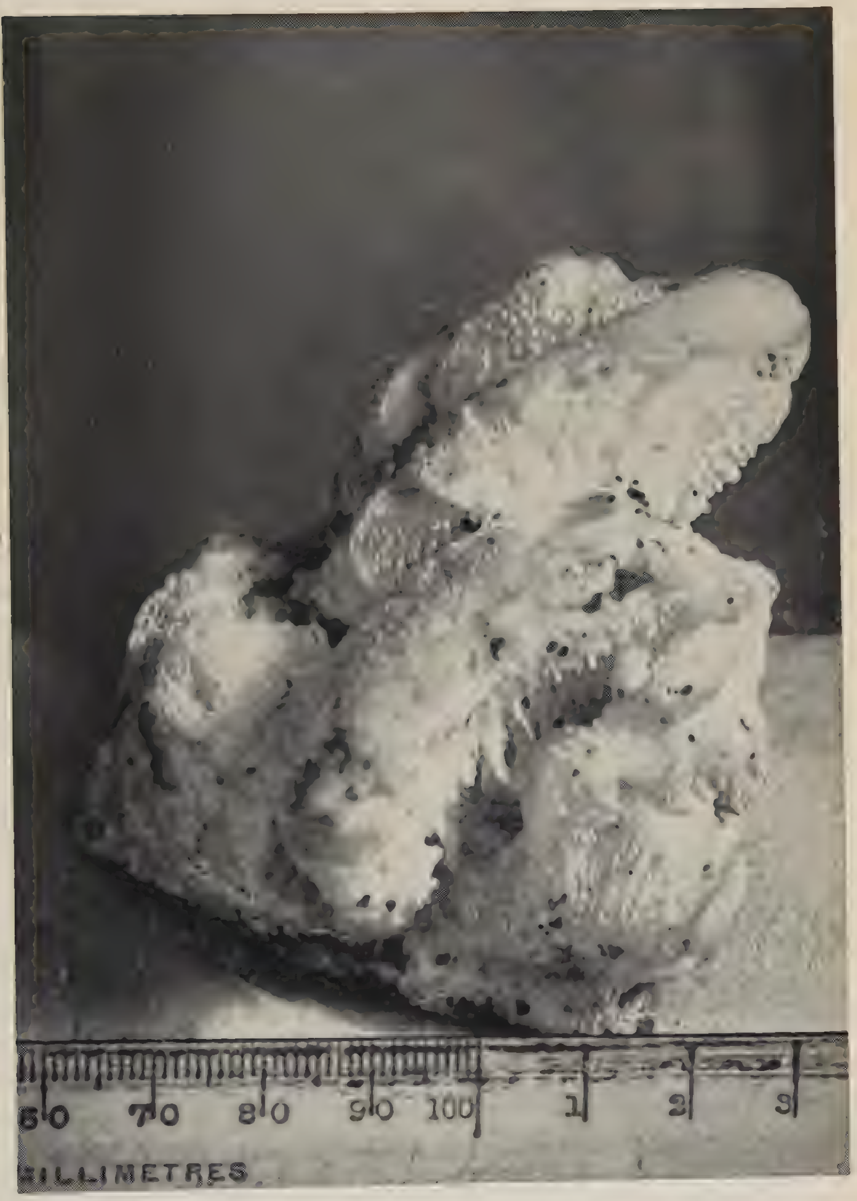
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NEW AND INTERESTING PLANT DISEASES

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37. Two Powdery Mildews on Rock Rose

On 8 September 1947 Miss J. M. Gooby brought us some mildewed leaves from a seedling rock rose (*Helianthemum vulgare*) growing in her garden in Harpenden. Some half a dozen other rock roses nearby were unaffected. Within twenty-four hours the same mildew, which proved to be *Leveillula taurica* (Lév.) Arn., was found on several varieties of *Helianthemum vulgare* in three other gardens in Harpenden, and later the same month or in October it was found by us at Exmouth (Devon) and Luton (Bedfordshire), by Dr A. S. Boughey at Exeter, and by Mr W. Buddin on *H. rhodanthe carnea* near Reading. Usually some varieties in the garden were affected, while others escaped: sometimes the mildew was found mainly on varieties with soft, broad leaves and sometimes on those with narrow, more wiry foliage. The fungus formed a dense white felt on the underside of the leaves and was also present to some extent, though much less conspicuously, on the upper surface. On microscopic examination, abundant branched mycelium was observed in the mesophyll and in the substomatal chambers. The conidiophores emerged singly or in twos or threes through the stomata, were simple or branched, and bore the conidia singly or in chains of up to six. The conidia were large and varied greatly in form, being long cylindric, elliptic, barrel-shaped or tapering towards one end. They measured 45–70 × 12–21 μ (average of thirty spores 56 × 17 μ) and usually showed clearly a small scar of attachment. Many of them had germinated in situ, with germ tubes 3 μ wide and up to 150 μ long, developed from near one end of the conidium. Cleistocarps were found on some of the leaves, embedded and often hidden in the felt of mycelium. They were bright yellow when young, but soon turned brown and ultimately black. When examined at the end of September many of the cleistocarps were black, cauldron-shaped or pezizoid, 90–200 μ in diameter (average of thirty = 130 μ), with numerous simple or branched mycelial-like appendages often with a knarled appearance, brown at the base, and hyaline at the tips. The contents were then not fully differentiated, but by the beginning of November, ripe ascospores were found in cleistocarps collected in Harpenden. The asci were not distinctly seen, but a number of ascospores, usually 8–10, occasionally more, measuring 33–42 × 16–19 μ (average of ten = 36 × 18 μ), were present in each cleistocarp.

According to Blumer (1933), *Leveillula taurica* is mainly distributed in the arid steppe regions of central Asia around the Black Sea and around the Mediterranean. He listed it on *Helianthemum canum* (L.) Baumg. in Czechoslovakia, on *H. oelandicum* Koch. in Yugoslavia, and on *H. vulgare* Gaertn. in Poland. It has not been recorded before in the British Isles, and its prominence in widely separated southern districts in 1947 may well have been closely related to the hot dry summer of that year.

On 10 November 1947, when examining plants of *Helianthemum* for *Leveillula* in the Royal Botanic Garden at Edinburgh, one of us (F. J. M.) found what appeared superficially to be this mildew on the undersides of the leaves of *Helianthemum pilosum purpureum*. On microscopic examination, however, the fungus proved to be different. The conidia corresponded in size and shape to those of most genera of the Erysiphaceae: they measured 24–30 × 15–18 μ (average of twenty-five was 29 × 15 μ) and contained fibrosin granules. No cleistocarps were observed and the fungus could not be definitely identified, but it was perhaps the imperfect state of *Sphaerotheca fuliginea* (Schlecht.) Salm., which has been recorded (Blumer, 1933) on *Helianthemum vulgare* Gaertn. in France and Switzerland.

Material of *Leveillula taurica* is deposited in the Herbarium of the Plant Pathology Laboratory at Harpenden as Herb. Path. Lab. Nos. 1–4, and Nos. 429–30, and that of the *Oidium* on *Helianthemum pilosum purpureum* as Herb. Path. Lab. No. 6.

38. DOWNY MILDEW OF ROCK ROSE (*PERONOSPORA LEPTOCLADA*)

On 14 October 1947, while looking for *Leveillula taurica* at Bloxham, near Banbury (Oxon), Mr W. Buddin collected what he thought at first to be this mildew, on the undersides of the leaves of some seedling rock roses. On microscopic examination, however, he found the fungus to be a species of *Peronospora*, and he very kindly sent us the material for deposition in Herb. Path. Lab. (as No. 5). We identified the fungus as *P. leptoclada* Sacc. in Michelia II, p. 530, with conidia 18–27 × 18–21 μ (average of thirty = 24 × 19 μ).

Gäumann (1923) distinguished two species of *Peronospora* on *Helianthemum*. One of these, found on *H. vulgare* Gars. and *H. guttatum* Mill., with conidia averaging 26 × 19 μ, he identified with *Peronospora leptoclada*: the other, on *Helianthemum alpestre* (Jacq.) Dunal. and *H. niloticum* Mönch., with more spherical and somewhat larger conidia averaging 27·8 × 23 μ, he named *Peronospora alpestris* Gäum. Nothing is known about the biological behaviour of these two fungi on the various species of *Helianthemum*, and the morphological differences between them seem to be very slight.

39. POWDERY MILDEW ON CHERRY LAUREL

Moore (1943) has referred to the occasional occurrence of an *Oidium* on cherry laurel (*Prunus lauro-cerasus* L.) in South Wales. Mr John Rees noticed a rather striking instance of it at Cardiff in July 1937, on a cherry laurel hedge. The hedge, which was continuous along the frontage of two houses, was almost wholly affected in front of one house and almost completely unaffected in front of the other. The difference was thought to have been related to a difference in the date of clipping the two parts of the hedge. In September 1938 slight attacks of the same mildew were seen again in various parts of Cardiff, as well as at Usk (Monmouthshire). In November of the following year, when the mildew developed on a number of frontage hedges in one road in Cardiff, Mr Rees kindly sent one of us (W. C. M.) specimens of it (Herb. Path. Lab. No. 28). The mildewed leaves were twisted and ruffled, and the upper surfaces showed indefinite chlorotic areas, which made the affected hedges look conspicuous. The undersides of the leaves were partly covered with a sparse weft of white or pinkish white mycelium. There were also some light reddish brown roughened areas on these leaves, but similar areas were found on a few leaves that showed no evident sign of the mildew. The conidia were borne in chains and when freshly mounted in water were cylindric, rounded at both ends, contained fibrosin bodies, and measured $30–41 \times 12–17\,\mu$ (average of fifty $= 34 \times 15\,\mu$). No cleistocarps were seen.

Powdery mildew is evidently not common on cherry laurel either here or abroad, and its identity is still uncertain. Cleistocarps have not been observed at all in Britain, and when Salmon (1906) previously found the *Oidium* here in 1905 on *Prunus lauro-cerasus* and its var. *camelliaefolia* in the Royal Botanic Gardens at Kew, he only assumed that it belonged to *Sphaerotheca pannosa*, chiefly because he found the persistent pannose patches of mycelium characteristic of that species on the young stems. Bertolini (1879) had been the first to describe an *Oidium* on this host: he observed it on cherry laurel fruits in Italy and named it *O. passerinii*. The following year, Roumeguère (1880) recorded *O. passerinii* from Tarbes in France, but he claimed to have found cleistocarps as well, each with four asci and five to seven brown spores, and he named the fungus *Erysiphe bertolini*, though according to Salmon (1906) it was distributed in Roumeguère's Exsiccati Fung. gall. No. 963 under the name *Oidium lauro-cerasi*. The mildew has also been identified—probably correctly—as *Podosphaera oxyacanthae* (DC.) de Bary var. *tridactyla* (Wallr.) Salm. Fischer (1919)* found both conidia and cleistocarps of that fungus in Switzerland. The cherry laurel bushes in the Botanic Garden at Berne were pruned hard following damage by frost during the winter of 1918–19, and mildew appeared in September 1919 on the new and still immature shoots that had developed during the summer. Fischer explained the comparative rarity of the disease on cherry laurel on the assumption that at the time when the *Podosphaera* usually appears on other species of *Prunus*, cherry laurel leaves are normally fully developed, hard, and unsusceptible.

40. *ALTERNARIA* STEM BLIGHT OF GODETIA (*ALTERNARIA GODETIAE* NEERGAARD)

Early in September 1947 a few dry, almost leafless, and apparently dead godetia plants were received at the Plant Pathology Laboratory from Feering, near Kelvedon in Essex. These plants were said to be suffering from a disease that had appeared rather suddenly two or three weeks previously, and that had progressed rapidly through certain stocks raised for seed purposes, particularly those of the varieties Sybil Sherwood and Kelvedon Glory. The specimens showed no obvious sign of a root disease or of having wilted, and their appearance suggested that they might be suffering from the effects of the severe summer drought that had prevailed. The stems were more or less blackened by a coating that consisted chiefly of the conidiophores and conidia of a species of *Alternaria*, whilst *Botrytis cinerea* was fruiting on the seed pods, buds, and remains of the petals, but both these fungi were regarded at the time as probably secondary. Pycnidia of *Diplodina passerinii* Allesch. were also noticed on one or two stems, though the typical symptoms of the disease caused by this fungus were lacking.

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* An article by van der Lek (1920) on mildew of cherry laurel merely summarizes Fischer's observations.

On 22 September one of us (W. C. M.) visited the affected field with Dr R. E. Taylor, who a few years previously had studied Wilt of godetia caused by *D. passerinii* (Taylor, 1941). The seed crop from a bed of Kelvedon Glory, about fifty yards by ten, was being harvested that day, though the yield was a very poor one, for most of the plants were prematurely brown and dry, the leaves had either fallen or they fell off as soon as they were touched, and the fruits contained little or no mature seed and were shed early. It was not difficult to find pycnidia of *Diplodina* on some of the plants, but the typical symptoms of Wilt, notably the characteristic rot at the base of the stem, were lacking. Nor did it appear probable that the premature withering of the plant was solely an effect of drought in what had been a hot and sunny summer. A few specimens, together with a sample of seed collected from withered plants, were taken back to Harpenden for closer examination. The same species of *Alternaria* as had occurred on the original specimens was present on the dead stems in soot-like patches consisting mainly of conidiophores, often fasciculate, bearing chains of spores. Most of the spores were beaked, and the beak sometimes terminated in a swollen tip. The total length of the spores, including the beak, ranged from 27 to 66 μ (average 46 μ): the body measured 18–48 × 9–18 μ (average of fifty = 34 × 13 μ) and the beak was 0–30 μ long (average 12 μ). The spores showed 3–8 transverse and 0–6 longitudinal septa. In tube and plate cultures on potato-dextrose agar there was at first a copious, white, aerial mycelium which soon became greyish green, while the submerged hyphae gradually became dark greenish black. Spores from cultures were very variable in size and most of them were unbeaked.

Thirty of the seeds brought back from Feering were placed on moist filter-paper in a dish on 29 September, and were examined about a fortnight later. Five of them failed to germinate: all these became covered with the *Alternaria* and on two of them *Botrytis cinerea* Fr. developed in addition. The rest had germinated, but all showed signs of disease, which either began from the hypocotyl or from a leaf to which the testa had become attached. Some were already dead, and none grew to be more than an inch high before succumbing. The *Alternaria* developed on or was isolated from twenty seedlings, while *Botrytis cinerea* was found on seven and cultured from nine seedlings.

Some months later we received commercial samples of seed of the two varieties Kelvedon Glory and Sybil Sherwood, said to have been harvested from infected and non-infected crops in the nursery at Feering. Seventy seeds of each variety from both crops were tested in a Copenhagen germinator. The seeds and seedlings were examined after eighteen days and the results obtained are shown in Table 1. The *Alternaria* developed on most of the seeds that failed to germinate, and also severely attacked many of the young seedlings produced. The seed from the crops not noticeably affected in the field was contaminated with the fungus, but only to about one-half the extent of the seed from the obviously infected crops.

The species of *Alternaria* concerned was identified as *A. godetiae* Neergaard (1945 *b*). This is the species which Neergaard (1938) described, under the name *Alternaria* sp., as the cause in Denmark of a disease obviously identical with that seen in 1947 in Essex. Neergaard noticed this disease first in 1937 and for the next few years it was severe at times on seed crops of godetia in Denmark (especially on the varieties Kelvedon Glory and Sybil Sherwood), often with the result that the yield of seed was reduced to a small fraction of the normal. In his monograph on Danish species of *Alternaria* and *Stemphylium*, Neergaard (1945 *a*) named the fungus *Alternaria tenuissima* (Fr.) Wiltsh. var. *godetiae* n.var., but later (1945 *b*) he raised it to specific rank as *A. godetiae*. He demonstrated the pathogenicity of the fungus (1945 *a*), showed it to be carried on the seed (1938–45), and obtained some measure of control by seed disinfection for half an hour in a ¼% solution of Uspulun (1938). Incidentally, Neergaard (1938–45) has also found *Botrytis cinerea* on godetia seed on a number of occasions, and has shown that it can be pathogenic to sterile seedlings and mature plants.

Table 1

	Seeds failing to germinate (%)	Seeds developing *Alternaria* (%)	Seedlings becoming infected (%)
Kelvedon Glory:			
Crop obviously infected	17	46	33
Crop not obviously infected	3	23	23
Sybil Sherwood:			
Crop obviously infected	8	47	40
Crop not obviously infected	6	27	24

Alternaria Stem Blight has not hitherto been seen in any country other than Denmark.

41. STEM ROT OF MELON (*MYCOSPHAERELLA CITRULLINA*)

About forty years ago, Grossenbacher (1909) investigated a disease of musk-melon (*Cucumis melo* L.) which had caused a good deal of damage in the greenhouses of the New York Agricultural Experiment Station, and he proved that this disease was caused by a fungus which he named *Mycosphaerella citrullina*. About the same time Massee (1909) received specimens of a stem rot of tomato from Waltham Cross and a similarly diseased cucumber plant from Gloucestershire. He found on both plants a species of *Ascochyta* which he regarded as identical with the imperfect stage of Grossenbacher's fungus, and as in a few preliminary inoculation experiments he apparently succeeded in infecting young tomato plants with spores taken from the cucumber plants, to say nothing of vegetable marrow with spores from tomato, he did not hesitate to attribute both diseases to *Mycosphaerella citrullina*, though he saw only the pycnidial state. Others (see e.g. Brooks & Price, 1913) followed Massee's example for a time, but ultimately Brooks and Searle (1921) showed that at any rate the tomato fruit rot, which had by then become widely distributed in England, was caused by the fungus now known as *Didymella* (*Diplodina*) *lycopersici*. Among the many strains of *Phoma*, *Ascochyta* and related fungi which they studied, was an authentic culture of *Mycosphaerella citrullina* (C. O. Smith) Grossenb. sent to them by Dr C. L. Shear in 1919, as well as two herbarium specimens of *M. citrullina* collected on cucumber fruits and water melon in Florida, and herbarium material collected in England under the same name from tomato and melon. After a very careful study of the different strains Brooks and Searle concluded that 'contrary to the usually accepted idea, the species *Mycosphaerella citrullina* (C. O. Sm.) Gross. has not yet been found in England'. They suggested that it should therefore be deleted from the list of known British species because 'the cultural characters and general behaviour of authentic specimens of *Mycosphaerella citrullina* from America do not agree with any of the fungi isolated from tomato in this country'. This view was generally accepted, the name *M. citrullina* ceased to be used in this country for a parasite of tomato and cucurbits, and the species does not find a place in the latest list of British Pyrenomycetes (Bisby & Mason, 1940).

Nevertheless, recent observations by us indicate that this fungus does occur in Britain. On 10 July 1947, Mr E. Skillman sent us a portion of the stem of a diseased melon grown at Hockley, Essex. The plant was one of a number thought to be suffering from a disease which had troubled the same grower some thirty years previously. Uniformly and thickly distributed over the piece of stem were numerous partially or wholly immersed, black, spherical perithecia, 135–200 μ in diameter (average of thirty-five = 170 μ), each with a clearly marked ostiole 24–27 μ across. The asci were fasciculate, aparaphysate, unthickened at the apex, 45–63 × 9–12 μ (average of twenty-five = 57 × 9 μ), and each contained eight hyaline ascospores. These were usually monoseriate, two-celled, with the distal cell nearly always larger than the other, oval with rounded ends, rather markedly constricted at the septum, and measuring 9–18 × 3–6·5 μ (average of seventy = 13 × 4·5 μ). Many spores were top-shaped, with the larger cell broadly ovate and the smaller tapering. Mixed with the perithecia were a number of what we took to be pycnidia, though we could find no spores in them.* They were somewhat irregular in shape, lighter in colour than the perithecia, and ranged in size from 85 × 75 to 128 × 110 μ (average of twenty = 113 × 96 μ).

This fungus agrees very closely with the description and figures of *M. citrullina* (C. O. Smith) Grossenb., with which we identify it. We have compared it with material of the perfect state of *Didymella lycopersici* as collected by Hickman (1944) on tomato at Long Ashton (Herb. Path. Lab.

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* In material from the same batch of plants examined at Cheshunt, Mrs Sheard found pycnidia measuring 91–128 × 87–120 μ (average 115 × 105 μ) and containing two-celled spores 9–13 × 4–6 μ (average 11 × 4 μ). In a few preliminary inoculation experiments Mrs Sheard obtained a rot of cucumber fruits but not of tomatoes with the fungus from melon, and she successfully infected tomatoes but not cucumber fruits with *Diplodina lycopersici* from tomato. Shortly afterwards she examined cucumbers affected with a stem rot caused by a fungus similar to that on the melon, and with this fungus successfully inoculated both cucumber and melon.

No. 96) and, apart from the aparaphysate nature of the perithecium which is a distinguishing feature of *Mycosphaerella*, there are distinct differences in the size and shape of the ascospores. Those of *Didymella lycopersici* are longer, more spindle-shaped and less constricted at the septum.

Mycosphaerella citrullina is not restricted to melon. Abroad, mainly in U.S.A. and Japan, it has also been held responsible (see, for example, Wiant, 1945) for a wilt or fruit black rot of *Cucumis sativus* L. (cucumber), *C. melo* L. (musk melon), *Citrullus vulgaris* Schrad. (water melon), *Cucurbita maxima* Duchesne (winter squash), *C. pepo* L. (summer squash, gourd, pumpkin, vegetable marrow), *C. moschata* Duchesne (Cushaw squash), and *Sechiumedule* (Jacq.) Swartz (chayote).

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THE EFFECT OF ADDING TRACE ELEMENTS TO CZAPEK-DOX MEDIUM

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Culture media containing only well-defined, chemically pure substances are widely used for studying fungi. For taxonomic work their chief advantage is their reproducibility by workers in different laboratories, whilst for biochemical studies of all kinds they are obviously more suitable than more complex media made from plant and animal extracts, even though the latter may be essential for maintaining vigour in stock cultures of many species.

Probably the most generally used synthetic medium is that devised by Czapek and modified by Dox, containing, per litre, sodium nitrate 2·0 g., potassium chloride 0·5 g., crystalline magnesium sulphate 0·5 g., potassium phosphate (either KH_2PO_4 or K_2HPO_4 according to the pH required) 1·0 g., crystalline ferrous sulphate 0·01 g., and any desirable source of carbon. In this laboratory Czapek-Dox medium, containing 3 % sucrose and 1·5 % agar, as recommended by Thom (1930), has been used for many years in routine examinations of species of *Aspergillus* and *Penicillium*, and the same basal medium but with 5 % glucose as source of carbon has been used for numerous biochemical studies. However, during the late war (there is no record of the exact date, but, for reasons given below, it was probably the summer of 1941) the agar medium suddenly changed in character, although, as far as could be ascertained, it was still made up from the same brands of pure chemicals, and from the same batch of agar, a large quantity of which had been purchased prior to the war. It was found that many species of *Penicillium* began to grow atypically, producing semi-floccose, pale brownish colonies, with tardy and sparse production of conidia. Sporing usually commenced only at the edges of colonies which had grown to fair size over a period of five or more days.

All the constituents of the medium were tested for possible toxic impurities, but nothing was found which could explain the change. Next a batch of medium was made up with tap water and tested alongside the usual medium made with distilled water. There was no appreciable difference between the two media with any of a large number of species tested. Similarly, additions of yeast extract or corn-steep liquor affected the growth of very few species.

It was then recollected that, prior to 1941, all the distilled water used for making culture media came from an old tinned copper still which had been in continuous use since 1929, and that it had frequently been reported from various departments of the School that the water contained traces of copper. The still was completely destroyed in May 1941 and a more modern type, subsequently installed, produced a distillate in which no trace of copper could be detected. It was therefore decided to test the effect of adding copper and other trace elements to the ordinary Czapek-Dox medium.

(*a*) *Addition of copper and zinc.* Four batches of medium were made, one containing no trace elements (except the usual iron), one containing 0·001 % $CuSO_4.5H_2O$, one with 0·001 % $ZuSO_4.7H_2O$, and the fourth with the same percentages of both salts. A number of species of *Penicillium*, all of which had been found to grow abnormally on the usual medium, were sown simultaneously on all four media. Both copper and zinc, used separately or together, had a marked stimulatory effect on spore production. In general, copper alone was better than zinc alone, and the combination, for a few species, better than either element used singly.

(*b*) *Other trace elements.* As a preliminary the mixture of trace elements used by Brian, Curtis and Hemming (1946), including iron, copper, zinc, manganese and molybdenum, was tried. There was slight stimulation of some species, but in general the results were much inferior to those obtained with copper and zinc in Exp. 1. The concentrations of trace elements in this mixture are apparently too low for maximum effect, iron and zinc being approximately 1/10, and copper 1/70, of the amounts used in Exp. 1.

(*c*) Five media were made and tested simultaneously on a number of species of *Penicillium*. They contained trace elements as follows: (1) Brian's mixture at ten times the recommended concentration; (2) similar but with increased copper; (3) as (2) but with addition of calcium as chloride; (4) copper and zinc alone; (5) none.

The actual percentage amounts of salts of trace elements in the four modified media, omitting iron which was present at the same concentration in all five media, were:

	(1)	(2)	(3)	(4)
Copper sulphate	0·00015	0·0005	0·0005	0·0005
Zinc sulphate	0·001	0·001	0·001	0·001
Manganese sulphate	0·0001	0·0001	0·0001	—
Potassium molybdate	0·0001	0·0001	0·0001	—
Calcium chloride	—	—	0·001	—

With all the species used cultures on (2), (3) and (4) were almost indistinguishable, and a great improvement on the cultures on ordinary Czapek-Dox. With some species, but not all, medium (1) was as good as the other trace element media, but in no case was it better. The results indicated that copper and zinc are the essential trace elements and that additions of small amounts of manganese, molybdenum and calcium have little if any effect.

(*d*) In order to find whether copper and zinc, used in the comparatively high concentrations as in medium (4) above, have any adverse effect, this medium was used for the routine subculturing of a collection of about 250 isolates of species of *Penicillium*. In no case was there any evidence of a toxic effect and with some sixty species there was marked improvement in freedom of spore production. The same medium was then used for growing several hundred species and strains of other genera of moulds. In many cases there was definite stimulation, and only with a few strains of yeasts was there indication of a slight toxic effect.

(*e*) In view of the observed effects of copper and zinc on spore production it was obviously of interest to test the effect of these elements on yields of metabolic products, since it has often been noted in these laboratories that the best yields of characteristic products are associated with rapid and typical growth. Very encouraging results have already been obtained with two species of *Penicillium*.

Citromycetin. This substance, first described by Hetherington and Raistrick (1931*a*), is produced by most strains of *P. glabrum* (Wehmer) Westling. This species was chosen because it had already been found that most strains grow atypically on ordinary Czapek-Dox and are markedly stimulated by additions of copper and zinc. Citromycetin is readily detected in culture fluids by adding ferric chloride solution, this giving a very intense green colour. With some strains of the mould the ferric chloride reaction, after about two weeks' incubation, is so intense that the solution, contained in an ordinary test-tube, is quite opaque and has to be diluted many times before becoming transparent. The relative amounts of citromycetin produced under different cultural conditions, or by different strains of the mould, may be roughly estimated by comparing the dilutions required to bring the solution to some arbitrary standard depth of colour.

Seven strains of *P. glabrum* were used for the experiment. Each strain was sown in four flasks of ordinary Czapek-Dox solution containing 5 % glucose (hereafter denoted as C-medium) and four flasks of the same medium to which was added 0·0005 % copper sulphate and 0·001 % zinc sulphate (CT-medium). The flasks were of one litre capacity and each contained 350 ml. of medium. All the flasks used for one strain were sown from as nearly as possible identical slopes and were incubated at 24° C. Flasks were harvested after five, eight, eleven and fifteen days' incubation.

Growth on CT-medium was much more rapid than on C-medium, as was also the consumption of sugar. For example, after eleven days' incubation residual glucose in the CT-medium varied, for different strains, from 1·3 to 1·6 %, and on C-medium from 3·0 to 3·9 %. On C-medium none of the strains gave, at any stage of incubation, more than a faint reaction for citromycetin. On CT-medium the worst strain gave a ferric chloride colour which was only just transparent, whilst the other six strains gave reactions ten to seventeen times as intense.

Citrinin. This yellow pigment was first isolated from *P. citrinum* Thom by Hetherington and Raistrick (1931*b*). It is also produced in good yield by *P. implicatum* Biourge (author's unpublished observation). It is readily isolated, as it is precipitated almost completely on acidification of the culture fluid. *P. implicatum* was sown on C- and CT-media (as described above), thirty flasks of each medium. It had been found previously that the yield of citrinin reaches a maximum when the apparent residual glucose (by polarimeter) has fallen to between 0·5 and 1·0 %. The cultures on CT-medium were harvested after nineteen days' incubation, when the apparent residual glucose was 0·66 %. The culture fluid, on acidification, gave 20·0 g. of good quality citrinin. The cultures on C-medium showed considerable variation. Of two flasks tested after nineteen days' incubation one had 1·13 % residual glucose and gave no precipitate on acidification, whilst the other had 1·32 % glucose and gave a fair amount of precipitate.

The cultures were finally harvested after twenty-seven days and gave only 5·9 g. of crude citrinin which was somewhat brownish and of inferior quality to that obtained from the CT-medium.

SUMMARY

1. Some moulds, particularly a number of species of *Penicillium*, grow atypically on Czapek-Dox medium made up with pure chemicals.

2. The medium is satisfactory if copper (0·0005 % $CuSO_4.5H_2O$) and zinc (0·001 % $ZnSO_4.7H_2O$) are added.

3. Addition of copper and zinc stimulates production of citromycetin by *P. glabrum* and citrinin by *P. implicatum*.

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A NEW PREDACIOUS SPECIES OF *TRICHOTHECIUM*

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(With 2 Text-figures)

In June 1947, in the course of investigations on the distribution of predacious fungi in decaying vegetable matter, a species of *Trichothecium* was found to be capturing and destroying nematodes in considerable quantity.

The fungus first appeared when sterile plates of maize-meal agar were inoculated with small quantities of vegetable compost supplied by Dr M. C. Rayner from composts prepared under her direction at Bedford College, London. A few days after inoculation it was observed that a number of rather straight, septate hyphae, mostly 3–5 μ in diameter, were growing out from the inoculum over the surface of the agar. These hyphae bore at intervals short lateral branches which, by curving back and anastomosing with the main hypha or with other similar branches, formed a series of three-dimensional networks (Fig. 1 *a*). The loops were usually 35–65 μ in external diameter and 25–40 μ in internal diameter, and the hyphae which formed them contained dense cytoplasm with numerous large vacuoles. The loops were very similar to those formed by *Arthrobotrys oligospora* Fres., as described by Zopf (1888) and others.

Nematodes moving over the surface of the agar were captured by adhesion to these loops, and in struggling to escape the animals often became entangled in the meshes of the networks, though such entanglement was by no means necessary for effective capture. For a short time the nematode would struggle violently, but in all observed instances they were held fast and, usually after about an hour, became still and apparently dead. It seemed clear that the nematodes were held by some sticky substance, though on no occasion was it possible to observe the presence of the viscous fluid which is often to be seen when eelworms are captured by *Arthrobotrys oligospora*.

Shortly after capture, while the nematode is still alive, its cuticle is penetrated by a short outgrowth from the loop to which it adheres, the outgrowth originating from the point of contact. This outgrowth expands within the nematode to form a bulbous structure (Fig. 1 *b*), usually 10–23 μ in diameter, from which trophic hyphae, usually 3·5–5·5 μ wide, grow out to fill the carcass of the eelworm and absorb its contents, so that eventually only the integument, filled with hyphae, remains. At first these trophic hyphae contain abundant cytoplasm, but later, when their work of absorption is completed, most of the cytoplasm is withdrawn.

A notable feature of the predacious activity of this fungus was the avidity with which nematodes were captured and consumed. They were often caught in groups of as many as a dozen, forming masses easily visible to the naked eye, and almost completely obscuring the networks which held them. A number of different species were captured, some of them of large size; the biggest measured was a specimen of *Rhabditis* 1·2 mm. long. The networks themselves, however, were often of comparatively small extent; on the whole they were less extensive than those normally formed by *Arthrobotrys oligospora.* Often some of the loops of a network stood perpendicular to the surface of the agar-like croquet hoops, a position which probably added to their efficiency.

The actual cause of death of the captured nematode is uncertain. The bulbous structure intruded by the fungus soon after capture does not itself seem to be sufficient to kill the animal in the rather short time observed between capture and final cessation of movement. This infection bulb seldom measures more than two-thirds of the diameter of the eelworm, and often less than half. Drechsler (1937) states that in the retiary series of predacious Hyphomycetes this 'mortiferous excrescence'—to use his terminology—is directly responsible for the death of the animal by cutting it physiologically in half. After extensive observations of *A. oligospora*, *A. musiformis* Drechsler and the fungus at present under discussion, I have found nothing to substantiate this view. It appears to me more probable that the primary function of the bulbous intrusion is to provide a surface from which trophic hyphae can grow out quickly and in quantity into the interior of the animal. Whether any toxic substance is produced by the fungus is a matter for experiment to determine, but in the absence of further knowledge of the functions of these structures, to refer to them as 'mortiferous excrescences' seems apt to be misleading. The term 'infection bulb' would appear more in accordance both with what is so far known of them, and with the original terminology of Zopf (1888).

After eelworms have been extensively captured over a period of several days, conidia are produced, usually very sparingly. The conidia are borne in terminal clusters at the apices of erect conidiophores, 50–200 μ long. The conidiophores are often branched, sometimes more than once, a head of spores being formed at the apex of each branch. At first a single conidium appears as a swelling at the apex of the conidiophore; this then bends over to one side and a second conidium is formed at a point on the conidiophore morphologically just below the first. This process is repeated several times, so that a cluster of ten or even more conidia may be carried on one conidiophore (Fig. 2*a*).

The conidia are ovoid, usually 27–37 μ long and 14–16 μ in greatest diameter, with a broadly rounded distal end and a very bluntly rounded proximal end, with no trace of any apiculum. They are usually divided by a transverse septum into two approximately equal cells, and are absolutely sessile (Fig. 2*b*). They germinate readily, germ tubes often being produced by both cells.

In addition to conidia, intercalary chlamydospores are formed in large numbers, especially in older cultures. These are usually nearly spherical, 24–32 μ in external diameter, but sometimes ellipsoidal, measuring 28–59 μ long and 15–29 μ wide. These chlamydospores have a wall about 2 μ thick, composed of at least two layers. Under laboratory conditions they are known to have remained viable for six months, germinating readily at the end of that time when placed on moist agar.

The fungus was isolated in pure culture by picking off conidia under the microscope, and made good growth on maize-meal agar and on glucose Czapek's agar, the growth on Czapek's agar being improved by the addition of organic nitrogen. In pure culture the hyphae grow in rather straight lines radiating from the point of inoculation, producing anastomosing lateral branches of somewhat smaller diameter than the main hyphae, but no loop systems. On adding living nematodes to such a culture, the networks are quickly formed, and eelworms are captured. Chlamydospores are produced in large numbers in pure culture, but formation of conidia is

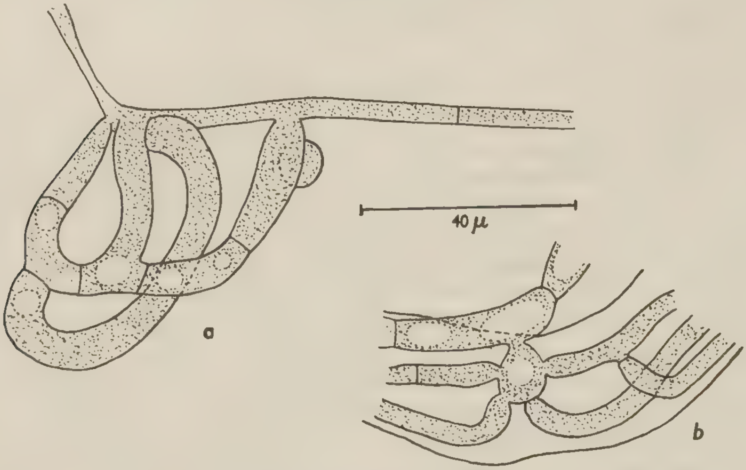

Fig. 1. *Trichothecium flagrans*. *a*, hyphal network; *b*, infection bulb within portion of eelworm, showing origin of trophic hyphae.

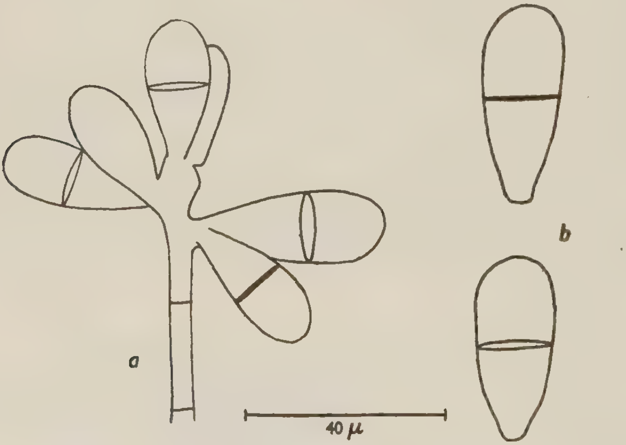

Fig. 2. *Trichothecium flagrans*. *a*, apex of conidiophore, bearing a cluster of six conidia; *b*, detached conidia.

very limited, and declines progressively during a series of transfers from plate to plate. Sporulation may be induced by culturing on rabbit-dung agar, even after the power to produce conidia has been apparently lost.

The morphology of this fungus, and especially the organs of capture, are suggestive of *Arthrobotrys*, but the shape of the conidia and the manner in which they formed in succession on conidiophores without sterigmata are more characteristic of *Trichothecium*. It is accordingly described as a new species of that genus, under the binomial *Trichothecium flagrans*, the epithet being chosen on account of the savagery with which it captures and consumes eelworms, as well as the large size of some of the nematodes with which it is able to cope.

Trichothecium flagrans sp.nov.

Mycelium expansum, hyphis (2) 3–5 μ latis sparse ramosis, spatiis 25–95 μ septatis; ramis lateralibus curvisque ad principem hypham atque inter se conjunctis qui reticula tri-dimensionis formant; laqueis singularibus (25) 35–65 μ latis extus, (12) 25–40 (47) μ intus; nematoda adhaesa in laqueis implicata capiens; bulbo infectionis 10–23 μ in diam. intrudente per integumentum nematodis e quo hyphae edentes 3·5–5·5 (6·5) μ latae excrescunt ad corpus implendum atque ad substantiam nematodis absorbendam. Conidia hyalina, continua effecta, quae caput 10 plus ad apices conidiophorum, saepe ramosorum, formant; ad altitudinem 50–200 μ supra substratum, 5·5–8·5 μ latum fundo, 3·5–5 (7) μ apice. Conidia sessilia, (20) 27–37 (45) μ longa, (11) 14–16 (17·5) μ in max. diam., apice proximo late globoso, (3·5) 4–6 (7) μ lato, plerumque per septum in duas partes circa aequas diviso. Mycelium etiam chlamydospores intercalarios efferens, plerumque subglobosos 24–32 μ in diam., subinde ellipsoideos plerumque (18) 28–59 μ longos, (15) 18–29 μ in diam.

Nematoda generis varii mensuraeque usque ad 1·2 mm. capiens atque edens. Hab. in retrimento plantarum e Bedford College, London.

Type material has been deposited in the Herbarium of the Commonwealth Mycological Institute, Kew, and in the Herbarium, Royal Botanic Gardens, Kew.

I should like to express my gratitude to Dr M. C. Rayner for supplying the sample of compost from which the collection was made, to Mr R. A. Gillanders for assistance in preparing the Latin diagnosis, and especially to Dr B. Barnes for valuable help at all stages of the work.

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OIDIOPSIS GOSSYPII (WAKEF.) RAYCHAUDHURI F. *INDICA* F.NOV. ON COTTON

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(With 2 Text-figures)

In December 1941, *Gossypium barbadense* Linn., grown at Dacca, was found to be affected by powdery mildew and about 11 % of the plants were infected with the fungus. Ashy white mycelial patches were observed on either side of the diseased leaf which turned yellow before leaf-fall. The disease was observed only on older leaves. The infection generally appeared late in the season and the diseased plants produced very few flowers and bolls.

The mycelium is hyaline, mostly superficial and borne on both sides of the leaf. Only a few hyphae penetrate the leaf through the stomata. These hyphae measure 4·5–6 μ in diameter. The conidiophores are simple, erect, septate, bearing broadly elliptical conidia. The conidia (Fig. 1) are vacuolate, granulate and borne singly at the tip; they measure 31–41 × 19–25 μ with an average size of 37·5 × 23·8 μ. Perithecia were not observed.

Microscopical examination of sections of diseased leaves has revealed that the mycelium penetrates through the mesophyll tissue on both sides of the lamina (Fig. 2).

Mildew of cotton has been recorded by several workers. Nowell (1923) records *Ovulariopsis gossypii* as causing a leaf mildew of Sea Island cotton in the Lesser Antilles. Butler (1918) reports the occasional occurrence of *Oidium* sp. on cotton in Bombay and suggests that the disease may be identical with the mildew *Oidiopsis taurica* (Lév.) Salm. (= *Erysiphe taurica* Lév.) of guar (*Cyamopsis psoraloides* DC.), while Abbott (1932) has expressed the opinion that *Ovulariopsis gossypii* is the imperfect stage of *Erysiphe malachrae* Seaver.

Zaprometov (1930) describes *Leveillula taurica* Arnaud f. *gossypii* on the upper surface of the leaves of two varieties of cotton, e.g. (i) Egyptian cotton 'Nubari', and (ii) upland type 'Navrotsky's Triumph' from Russian Central Asia. On the former variety of host the disease at its first appearance is distributed in separate angular portions 1·5–2 mm. in diameter which later coalesce into a continuous tomentose covering, while on the latter, the white tomentose mealy layer covers the leaf in separate irregularly rounded scattered spots. The conidia measure 43–50 × 13 μ and the perithecia have a diameter of 157–175 μ.

Wakefield (1920), however, found only the conidial stage of the fungus on Sea Island cotton (*Gossypium barbadense* Linn.) and named it *Ovulariopsis gossypii* Wakefield. The conidial measurements as found by her are 50–60 × 16–22 μ. In a personal communication she writes that the Indian fungus is a distinct form from that described by her, in having shorter and broader conidia. Moreover, she is now of the opinion that the fungus should be placed under genus *Oidiopsis* and not *Ovulariopsis* as described by her in 1920. Arnaud (1921) defined *Ovulariopsis* Pat. & Har. as being the conidial form of *Phyllactinia*, and characterized by having a single terminal club-shaped conidium and mycelium for the most part superficial. *Oidiopsis* Scalia he distinguished as the conidial form of *Erysiphe taurica*, for which he created the new genus *Leveillula*. In this fungus the mycelium is developed chiefly within the leaf, only after showing some external mycelium.

The Indian form of the fungus showed only the conidial stage. No perithecia were found on the host. The conidia differed from those of *Leveillula taurica* Arnaud f. *gossypii* in being shorter in length and broader in width.

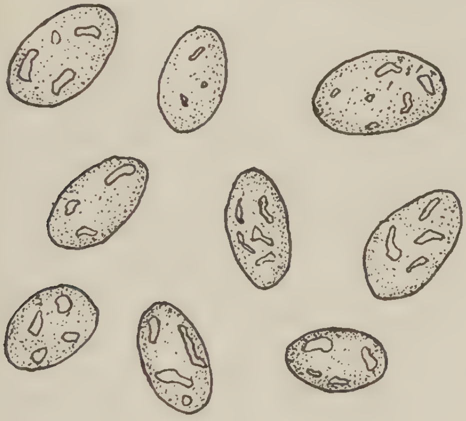

Fig. 1

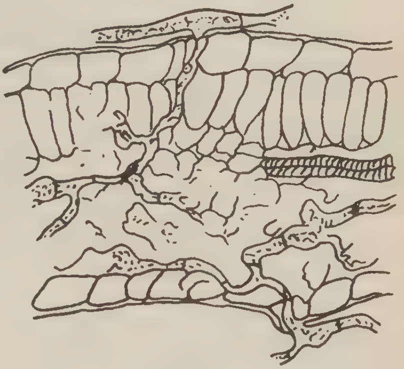

Fig. 2

Fig. 1. Conidia of *Oidiopsis gossypii* (Wakef.) Raychaudhuri f. *indica* f.nov. × 380.

Fig. 2. Transverse section of a part of infected cotton leaf showing the mycelium of *O. gossypii* (Wakef.) Raychaudhuri f. *indica* f.nov. within the mesophyll tissue. × 48.

In the light of Miss Wakefield's communication and as suggested by her, a new combination for the conidial form is proposed, e.g. **Oidiopsis gossypii** (Wakef.) Raychaudhuri nov.comb. Due to the marked difference in conidial measurements of this fungus a new form is proposed. The fungus is hence named **Oidiopsis gossypii** (Wakef.) Raychaudhuri f. **indica** f.nov.

Latin diagnosis. Mycelium hyalinum, fere omnino superficiale, in utraque facie foliorum; nonnullae tantum hyphae e stomatibus exeuntes; hyphae 4·5–6 μ diameter. Conidiophora simplicia, erecta, septata, late ellipticis conidiis ornata. Conidia vacuolata, granulata, apicalia, solitaria, 37·5 × 23·8 μ (31–41 × 19–25 μ). Hab. in foliis maturis *Gossypii barbadensis* Linn. Dacca, Bengal, December 1941, leg. S. P. Raychaudhuri (Typus). Typus in herbario Kewensis.

I am grateful to Miss E. M. Wakefield, Kew Herbarium, for taking much interest in the diagnosis of the fungus and to Dr R. W. G. Dennis, Royal Botanic Gardens, Kew, for sending a photostatic copy of a portion

of Zaprometov's paper. My thanks are due to Rev. Father H. Santapau of St Xavier's College, Bombay (now in England) for writing the Latin diagnosis.

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THE *BOTRYTIS* DISEASE OF GLADIOLUS WITH SPECIAL REFERENCE TO THE CAUSAL ORGANISM

By J. W. L. PEIRIS, Ph.D.

The work to be described in this paper arose as a sequel to the investigations of Dr L. E. Hawker of the Plant Pathology Laboratory, Imperial College, who in the years 1944 and 1946 published three papers relating to various fungus diseases of gladiolus. The last of these papers (Hawker, 1946) dealt with the *Botrytis* disease. The object of the present investigation was to explore the latter more fully, with special reference to the identity of the causal organism.

Plant Material and General Experimental Methods

This investigation was begun in 1945 with a stock of about 1200 corms left by Dr Hawker from her experiments and consisting mainly of 'Lilac Wonder', a rather robust and prolific variety. There were also small quantities of the varieties 'Van Tienhoven', 'Wolfgang', 'Flaming Sword', 'Rose Precoce', etc. suitable only for small experiments. Although it was thought desirable to have carried out experiments with a number of varieties, it was not possible under the existing wartime conditions to supplement the continually dwindling stocks and most of the work had to be confined to the variety 'Lilac Wonder'.

The corms were grown and harvested at the Field Experiment Station, Slough, and stored in an unheated shed over winter. They were cleaned two or three weeks after harvesting unless otherwise stated, and placed in shallow trays during storage. The diseased corms in the experimental lots were recorded at intervals of approximately ten days during storage. The healthy corms were planted out in March–April in the usual manner and the conditions of growth and flowering observed.

The experiments consisted of isolations from diseased plant material, inoculation of plants in the field and in storage, lifting and cleaning corms at different periods, and storage under various conditions.

Earlier Accounts of the Disease

The *Botrytis* disease of gladiolus was first reported in 1927 from Canada by Drayton (1928), and observed in the same year by Moore (1939) in England. From then onwards it has been reported as follows: by Drayton (1929) from Holland in 1929; by Van Poeteren (1938) from the same country with a statement that it was becoming more prevalent; by Dodge and Laskaris (1941) from Long Island, N.Y.; by Noble, Hynes, McCleery & Birmingham (1935) as attacking stems and leaves in New South Wales; by Dimock (1940) as causing a leaf and flower blight in Florida. The three most recent works on the subject are by Timmermans (1941, 1942) in Holland, Wade (1945) in Australia and Hawker (1946) in England.

In many of these papers there was no attempt to identify the causal fungus critically. There was no doubt of the presence of a *Botrytis*, but no specific determination was made. Some authors assumed it to be *B. cinerea*. Klebahn (1930) was the first to study the fungus in some detail. His conclusion was that it was morphologically different from *B. cinerea* and accordingly he named it *B. gladioli* Kleb. Klebahn's paper, however, contains no description of the disease. The later work of Timmermans (1942) showed that the causal fungus was different from the one described by Klebahn; hence the new species *B. gladiolorum* Timmermans. Hawker's work, it should be added, was concerned mainly with the control of the disease; she assumed the fungus to be *B. cinerea* and stated that it was similar in spore size to the fungus described by Moore (1939).

SYMPTOMS OF THE DISEASE

Although the *Botrytis* rot of gladiolus is most conspicuous and becomes most destructive on the corms during storage, it is, nevertheless, capable of attacking the plant at any stage of its life history. The following account gives my experience of the disease manifestations and is in substantial agreement with that given by Moore.

(*a*) *Symptoms during early growth.* The commonest cause of gaps in the rows is the planting of corms affected by *Botrytis*. Such affected corms may be passed as sound on superficial examination even when the scales are removed, as the interior of the corm may to some extent be attacked without any external symptoms. Infected corms put out a shoot which sooner or later becomes attacked by the fungus growing out from the corm, and causing the foliage to turn yellow and wither away. Sclerotia are often produced at the collar region of the plant and Wade (1945) has described this as the collar-rot stage of the disease.

In addition to the above mode of attack young plants arising from clean corms are sometimes infected from the soil and show a failure of growth after the shoot has elongated a few inches. In these plants, sclerotia and mycelium are generally to be found in the collar region and isolations give a species of *Botrytis* as the causal agent. During the last two years losses due to such early attack have arisen mainly from the planting of diseased corms. Infection of healthy corms has been comparatively rare, probably because fresh ground has been used for planting.

(*b*) *Symptoms on flowers.* When the flowering period is well advanced, and especially if some of the flower stalks have not been cut early enough, first the older flowers at the base of the stalk, and later the younger ones, become profusely covered with conidia and, if the weather is humid, this is followed by strong development of aerial mycelium. In the red-flowered variety 'Van Tienhoven' and even in such a pale-coloured variety as 'Lilac Wonder' initial attack in the petals may be apparent as pale white or water-soaked spots or areas. The abundant conidial production on flowers is interesting because the species of *Botrytis* responsible for *Botrytis* rot of gladiolus is peculiar as it does not normally produce conidia readily on artificial media. Wade (1945) made the sound observation that flowers left in the field contribute most of the spore load of this species of *Botrytis*, apart from causing direct loss by destruction of blooms.

(*c*) *Symptoms on leaves.* Leaves surrounding infected flowers may show numerous minute brown spots with definite reddish margins, or the spots may coalesce into larger blotches. From the latter the fungus is readily isolated, but from the former only occasionally; in this respect my experience agrees with that of Moore (1939) and of Wade (1945).

(*d*) *Symptoms on corms.* Moore (1939) was the first to recognize three forms of symptoms on the corms, as follows:

(1) Sunken rounded lesions, usually with a small cavity under the tightly stretched skin of the corm, a type of rot frequent in Canada but rarely seen in England. This is an arrested type of lesion, and there are good grounds for believing that English conditions are usually not warm enough to produce this localization of attack.

(2) 'Core-rot', commonly met with in England, leading to rotting and often to disappearance of the central tissue of the corm, which may thus appear as a ring.

(3) 'Spongy rot', affecting the whole tissue of the corm. This type of rot has been the commonest of all in my experience and every intergrade between this and core-rot has been seen. In advanced stages and under humid conditions, there is profuse mycelial development over the surface, and later the formation of large black coralloid masses of sclerotia. Small groups of conidiophores also occur on the sclerotia or on the general mycelium. The affected corms finally may become mummified.

ISOLATIONS

These were made from corms, leaves, flowers, and flower stalks of gladiolus attacked by *Botrytis* and gave the data which are collected in Table 1. Occasionally the isolates of *Botrytis* were accompanied by other fungi, e.g. *Penicillium*; the latter were disregarded. When, however, no *Botrytis* appeared, the other fungus was recorded, as shown in the last column of Table 1.

Table 1

			Botrytis		
Source of isolate	Description	No. of isolates	Mycelial type	Conidial type	Other fungi
Corms	Showing spongy rot	32	24	4	2 *Penicillium* 2 *Fusarium*
	Showing core and spongy rot	6	6	0	—
	Showing core rot	20	20	0	—
	Lesions on surface	5	4	0	1 *Fusarium*
Flowers	Covered with conidia	25	19	6	—
Leaves	Leaf blotches and specks	10	6	4	—
Flower stalks	Diseased vascular bundles from inside	25	20	5	—
		123	99	19	5

Table 1 shows that a mycelial type of *Botrytis* was consistently isolated from various diseased parts of the plant. These mycelial isolates were similar in their general habit of growth and, as will be shown later, belong to *B. gladiolorum* Timm. (1942), and they will be referred to as such. Isolates of *B. cinerea* were also obtained but the proportion of *B. gladiolorum* to *B. cinerea* was very high, namely, 99 to 19. It was thus early recognized that the species of *Botrytis* associated with the *Botrytis* rot of gladiolus was different from the usual species *B. cinerea*. The morphology, physiology and identity of the causal organism will be dealt with in a later part of this paper. No experiments have been carried out with the species of *Penicillium* or *Fusarium* which are noted in the last column of Table 1.

INOCULATION EXPERIMENTS ON GLADIOLUS

(*a*) *Corms*. Isolates of *Botrytis cinerea* from gladiolus and of *B. gladiolorum* were inoculated into corms which had been wounded at side, apex or base. Under the cool or cold conditions of winter storage the proportion of successful infections with *B. gladiolorum* was moderate: twenty-nine out of forty-four in one experiment and twenty-seven out of forty-one in another. Only seldom, for example in eight out of fifty-three samples, were unwounded corms infected. Under the same conditions inoculations with isolates of *B. cinerea* invariably failed. Moore and other workers have recorded similar experiences.

Temperature has a marked controlling effect on attack, as is shown by the data of Table 2.

Table 2

Temperature (° C.)	No. inoculated	No. infected
5–10	20	10 (slightly)
10–20	41	18
15	24	21
20–25	31	3

Inoculations at 15° C. consistently gave a high proportion of successful infections, and this result has been confirmed by the following experiment. Disks of gladiolus corm were inoculated at the centre with a small piece of mycelium of *B. gladiolorum* and stored under moist sterile conditions at temperatures of 5–10, 15, 20, 25 and 30°. Weights (g.) of rotted tissue produced in four days were as follows:

Temperature (° C.)	Weight (g.)
5–10	0·9
15	1·1
20	0·3
25	0·15
30	0

The greatest weight of rot produced was at 15° with a sharp drop as the temperature rose. On the other hand, the optimum for linear growth of the fungus was found to be about 22°, i.e. at a point where rotting of tissue is very slight. This is because gladiolus tissue readily forms cork at higher temperatures, as had been observed in the inoculation experiments already mentioned. At about 15° C. cork formation is sufficiently slow to enable the fungus to advance and hence the observed optimum for the development of the rot. Below 15° C., although cork formation is weak, the growth of the fungus is also retarded, and this accounts for the slight spread of the rot in this temperature range which is apparent for example in Table 2.

It is not unlikely that the optimum temperature for attack may be slightly different in different varieties according to their cork-forming capacities. The temperature of 15° C. was optimum for 'Lilac Wonder', but no other varieties were tried. It is therefore probable that varietal susceptibility to storage rot is at least partly determined by periderm-forming tendencies.

The presence of a low optimum for development of storage rot agrees with the general observation during this investigation that storage rot is essentially a low temperature disease. Drayton (1929) also observed that a cool temperature is favourable for the decay of corms. This view is supported by the good control of the disease obtained by high temperature storage to be referred to later.

(*b*) *Flowers*. Isolations from diseased flowers yielded a much higher proportion of *B. gladiolorum* than of *B. cinerea*. A series of inoculation experiments with flowers in moist chambers gave the results summarized in Table 3. The spores were applied in water either in the form of a fine spray or in rain-drop size.

Table 3

Isolate		No. inoculated	No. infected
B. gladiolorum (from naturally infected flowers)		33	32
		18	18
B. cinerea	Isolate I	33	9
	Isolate II	33	5

These results indicate clearly the greater pathogenicity of *B. gladiolorum*.

(*c*) *Leaves and shoots*. Table 4 gives the results of an experiment in which pieces of healthy leaves, after surface sterilization, were inoculated without wounding in a moist container.

Table 4

Isolate	No. of inoculations	No. of infections after			
		24 hr.	48 hr.	72 hr.	96 hr.
B. gladiolorum	20	4	4 definite, 4 slight	11 definite	12 definite, 4 slight
B. cinerea Isolate I	20	0	0	0	1 slight
Isolate II	20	0	0	2 slight	2 definite, 1 slight
Control	20	0	0	0	0

The effect of *B. gladiolorum* was apparent sooner, and the total number of infections was greater than with isolates of *B. cinerea*. The mycelium of *B. gladiolorum* was observed to penetrate right through the leaf to the other side. Rusty specks appeared at the margin of the lesion and were evidently caused at points of penetration of hyphae at an incipient stage of attack. These were very characteristic of *B. gladiolorum* and to a much less extent of the other strains.

In another experiment young gladiolus plants about 1 ft. tall, grown in moist sand, were inoculated on the leaves from agar cultures of

B. gladiolorum and *B. cinerea*. High humidity was maintained by covering the plants with bell jars. Here again rusty specks developed which coalesced into spreading lesions in the inoculations with *B. gladiolorum*. *B. cinerea* showed only a comparatively low degree of virulence (Table 5).

Table 5

Isolate	No. of plants	No. of leaves inoculated	Attack after 1 week	Attack after 2 weeks
B. gladiolorum	10	14	10	14
B. cinerea Isolate I	10	15	0	2
Control (agar only)	10	15	0	0

Somewhat more normal conditions were obtained in an experiment in which corms were allowed to grow in pots of sterilized soil in the greenhouse until almost the stage of flowering. Two healthy corms were planted in each of ten pots and at the time of inoculation the plants were nearly 30 in. tall. The inoculum, which consisted of a piece of an agar culture of *B. gladiolorum*, was placed in the axil of the second leaf above the soil. Sixteen shoots arising in five pots were thus inoculated, while fifteen shoots in the remaining five pots were used as controls. All the pots were placed in a humidity chamber for five days after inoculation, and then returned to the open air of the greenhouse.

Within a week, water-soaked lesions developed on some of the shoots of the inoculated series, and at the end of a fortnight every inoculated shoot showed spreading lesions which mostly developed downwards and round the shoot. The controls meanwhile remained healthy. In three weeks fourteen out of sixteen shoots of the inoculated series were so rotted that the tops generally buckled over at the point of inoculation. At the end of a month the rot had spread to the corms, which became soft, and typical conidiophores of *Botrytis* were seen at the collar region and on the apex of the corms.

Similar inoculations were carried out under field conditions. In September 1945, three types of inoculum were used, viz. *B. gladiolorum*, *B. cinerea* (eight-day-old cultures of each) and a one-month-old culture of the latter. Plants were inoculated one month before lifting, in the leaf axil just above ground level as in the above experiment, and controls were inoculated with sterile agar. Seventy-two shoots were used for each treatment. Table 6 shows the results.

Table 6

Isolate	No. of shoots visibly diseased after 10 days	Losses during growth	Percentage of corms rotted in store
B. gladiolorum (8-day-old culture)	52	2 *Botrytis*	58·3
B. cinerea (8-day-old culture)	4	1 *Botrytis*	51·4
B. cinerea (1-month-old culture)	1	3 *Sclerotinia*	44·9
Control (agar only)	0	1 *Botrytis*	48·6

Table 6 shows that within ten days a high proportion of the plants (viz. fifty-two out of seventy-two) inoculated with *B. gladiolorum* developed discoloured lesions at the point of inoculation, while the figures were four out of seventy-two for the young and one out of seventy-two for the old culture of *B. cinerea.* However, this high proportion of initial attack was not reflected in disease incidence in subsequent storage. The loss in storage of the lot inoculated with *B. gladiolorum* was not appreciably greater than of those inoculated with *B. cinerea* or of the controls.

In September 1946 a similar series of inoculations was made in the leaf axils with *B. gladiolorum* and with two isolates of *B. cinerea.* The percentage of successful inoculations was neither so great nor were the lesions so distinct as in the previous year, probably due to the lack of favourable weather conditions at the time. The corms were lifted a month afterwards, cleaned and stored in the usual manner and examined for *Botrytis* rot at the end of ten weeks (Table 7).

Table 7

Isolate	No. of shoots inoculated	No. of new corms	No. rotted 10 weeks after lifting	Total no. rotted in storage	Percentage of rotted corms
B. gladiolorum	60	60	4	54	90
B. cinerea **Isolate I**	60	60	2	40	67
B. cinerea **Isolate II**	60	60	0	39	65
Control (agar only)	60	60	2	45	75

Here again the increased incidence of corm rot arising from inoculating the shoots with *B. gladiolorum* was slight.

Similar results were obtained where inoculations were made, in September 1946, at the cut ends of the flowering shoots. Five weeks before lifting, the plants were cut 4–5 in. from the ground and inoculated with the same isolates as in the previous experiment, drops of sterile water being added occasionally to keep the inoculum moist. The resulting number of corms rotting in storage ten weeks after lifting and at the end of the storage period are set out in Table 8.

Table 8

Isolate	No. of shoots inoculated	No. of new corms	No. rotted 10 weeks after lifting	Total no. rotted in storage	Percentage of rotted corms
B. gladiolorum	25	25	3	25	100
B. cinerea **Isolate I**	25	25	1	24	96
B. cinerea **Isolate II**	25	25	2	22	88
Control (agar only)	40	40	0	35	87

It is probable that in the field experiments mentioned above, ideal conditions for infection or further spread of the fungus or for both did not prevail. This is particularly noticeable in the experiments for 1945 and 1946 where axils of plants were inoculated (Tables 6 and 7). Whereas in 1945 the parts above ground were strongly attacked but advance into the corm was poor, in 1946 even foliage infection was very slight. A study of rainfall figures shows that though the total precipitation for the fortnight following inoculation in the two years was not quantitatively different, it was more evenly distributed in 1945. The possibility of loss in vigour of the isolate of *B. gladiolorum* can be eliminated because greenhouse experiments carried out at about the same period as the above experiments were very successful.

As far as is known there are no other reports of inoculations of gladiolus plants in the field at a post-flowering stage with *Botrytis*. Wade (1945), however, obtained typical *Botrytis* rot in corms that were inoculated at the cut ends after digging and enclosed in tins, but none in those inoculated and stored on a wire-netting stretcher. It is also interesting in this connexion to note that although he produced heavy infection of leaves by brushing them with conidia, no *Botrytis* rot subsequently developed in storage.

Timmermans (1941) considered that infection travels along the vascular bundles into the corms, and suggested that when the latter could not be stored at a high temperature the stalks should be torn off immediately after lifting and not cut off at about 1 in. distance, as is the usual practice. An experiment carried out in the autumn of 1946 to test the value of this method did not give promising results, and in fact there was a higher percentage of losses in the batch treated according to Wade's suggestion. Thus, except possibly under special circumstances, as for example when heavy foliage infection occurs, there is no advantage in breaking off the tops of corms immediately after lifting. On the contrary, this might expose the corms to easier invasion by *Botrytis* and other fungi through the uncallused wound on the corm thus left exposed. It is therefore reasonable to conclude that infection occurs to a large extent either through the cut end or through the scar at the apex. Basal infection through the old corm is also evident though this, according to observations, is not as frequent as that from the apex. Natural infection through the basal scar after cleaning is to some extent another point of entry.

The following general conclusions are to be drawn from the preceding experiments:

(1) *B. gladiolorum* is capable of quickly setting up infection on growing gladiolus plants and, under favourable controlled conditions such as are obtained in a greenhouse, travels down to the corm and sets up rot in it.

(2) In the fluctuating environment of the field *B. gladiolorum* is much more pathogenic than isolates of *B. cinerea*. Although plants inoculated with *B. gladiolorum* gave a higher number of diseased corms in storage than did the controls and those inoculated with *B. cinerea*, this difference, however, was small. A high proportion of foliage infection in the field was not associated with a correspondingly high disease incidence in storage. This is probably because favourable conditions for infection and spread of the fungus in the field were not realized during these experiments.

(3) Irrespective of the infection of the shoot and transmission of the disease through the shoot into the corm, much rot always occurred in storage. The obvious interpretation is that this is due to natural infection of corms after lifting, either through the cut end of the flowering stalk after trimming or through the stem scars left at the top and bottom of the corm at cleaning. The leaf scars which occur round the corms are probably of little importance, inasmuch as side lesions are far less frequent.

EFFECT OF CULTURAL AND STORAGE CONDITIONS ON ROTTING OF CORMS

The experiments in this connexion follow the lines of earlier work by Hawker (1946) and substantially confirm her findings.

A number of rows of plants growing in the field were protected from wetting and a similar number were watered in addition to receiving the natural rainfall. These treatments were maintained for the three weeks preceding lifting. The percentage losses from *Botrytis* in the stored corms were 24 and 47 % in the batches from dry and wet soil respectively.

The effect of dates of lifting and cleaning is shown in Table 9.

Table 9

Date of lifting	Condition of soil at lifting	Date of cleaning	Percentage rotted
12 October	Dry	1 November	12
6 November	Very wet	20 November	16·4
22 November	Very wet	12 December	64·2
12 October	Dry	10 January	54·1
6 November	Very wet	10 January	95·4
22 November	Very wet	10 January	90

It is evident from Table 9 that when the plants are lifted early, storage losses are low, while the later they are left in the ground the more disease in storage increases. This is probably due to longer exposure to infection, to the greater tendency for the ground to get wet as the season advances, and to the slower rate of drying of the later lifted corms owing to the comparatively low temperatures prevailing at lifting.

The effect of conditions during the early period of storage is shown in Table 10.

Table 10

Conditions of storage during first month	Temperature (° C.)	Percentage diseased	
		Botrytis	Other fungi
Cool and moist	12–15	70	None
Cool and dry	12–15	46	6 *Septoria*, 2 *Sclerotinia*
Warm and moist	17–20	20	2 *Sclerotinia*, 4 *Penicillium* and *Sclerotinia*, 6 *Septoria*
Warm and dry	17–22	8	18 *Sclerotinia*, 4 *Penicillium*

It is clear from Table 10 that storage for one month at high temperature was very effective in the control of *Botrytis* disease. Warm dry conditions were better than warm moist conditions, emphasizing the necessity of good ventilation as well. The greatest number of diseased corms was obtained under cool moist conditions, and this agrees with the results of the inoculation experiments mentioned earlier, which showed that low temperatures favoured the disease. On the other hand, it is likely that other fungal troubles were increased by the warmer storage conditions, as shown in the last column of Table 10. From a similar experiment, Hawker (1946) concluded that the control of the disease by this means was due to the increased rate of drying of corms and the formation of callus over wounds. Timmermans (1941) also emphasized that storing immediately after lifting at 25–30° C. gives good control of *Botrytis* rot.

INOCULATION EXPERIMENTS WITH *BOTRYTIS GLADIOLORUM* AND STRAINS OF *BOTRYTIS CINEREA* ON OTHER HOSTS

In this work, four isolates of *B. cinerea* were used:

Isolate I. A conidial strain from gladiolus (GI).
II. A conidial-sclerotial strain from gladiolus (GII).
III. A conidial-sclerotial strain from lettuce (L).
IV. A conidial-sclerotial strain from daffodil leaves (D).

Inoculations were made in a standardized manner into roots of swede and turnip, and on lettuce leaves; also, for comparison, into gladiolus corms and on gladiolus leaves. With swede, turnip and gladiolus corms, the amount of rotted tissue was weighed, and as the inoculations were replicated, results were obtained which could be treated statistically. The results are summarized in Table 11.

Table 11

Host	Fungus	Replications	Weight of rot (g.)
Turnip	*B. gladiolorum*	10	0·04
	B. cinerea (G I)	10	0·27
	(G II)	10	1·63
	(L)	10	2·11
	(D)	10	0·67
Swede	*B. gladiolorum*	6	0·01
	B. cinerea (G I)	6	2·91
	(G II)	6	3·71
	(L)	6	4·52
	(D)	6	3·00
Gladiolus	*B. gladiolorum*	7	0·89
	B. cinerea (G I)	7	0·14
	(G II)	7	0·11
	(L)	7	0·13
	(D)	7	0·13

The differences in amount of attack between *B. gladiolorum* and any of the *B. cinerea* strains are highly significant on all three hosts, but whereas *B. gladiolorum* is the active fungus on gladiolus corms, it is much the weakest on the other two hosts, and conversely for the four strains of *B. cinerea*. The results with lettuce and gladiolus leaves, though less susceptible of quantitative treatment, were of a similar type. There is thus a clear distinction in pathogenic behaviour between *B. gladiolorum* and four isolates, chosen more or less at random, of *B. cinerea*.

CONIDIAL PRODUCTION OF *BOTRYTIS GLADIOLORUM*

In a comprehensive series of experiments (not included here) on the effect of cultural and environmental factors on the growth characters of *B. cinerea* (several strains) and *B. gladiolorum* it was found that the latter consistently produced very few conidia. This observation agrees with those of Wade (1945) and Dodge and Laskaris (1941) who found that the isolate of *Botrytis* obtained by them as the causal agent of *Botrytis* rot sporulated weakly in culture; also with Timmermans's (1942) finding that *B. gladiolorum* is a poor conidial producer in culture. Nevertheless, it was shown above that conidia are formed freely on flowers and to a less extent on flower shoots, leaves and corms of gladiolus. Formation of conidia in nature is obviously essential if the view is to be maintained that the predominant species of *Botrytis* in causing gladiolus *Botrytis* rot is *B. gladiolorum*. Special attention was therefore given to this subject.

Illumination of fairly strong intensity appears to be required for the sporulation of *B. gladiolorum*. Thus spores were freely produced on fresh flowers in Petri dishes when kept in front of the laboratory window in summer time, but not when kept in the dark.

Petri dishes containing a glucose-peptone medium (glucose, 0·5 %; peptone, 0·6 %, with mineral salts) were inoculated and placed (*a*) in front of a window under direct illumination for about four hours a day, (*b*) in diffused light, and (*c*) in the dark. At the end of four days normal erect grey conidiophores developed in the dishes in direct light but not in the other two sets. Under the conditions of (*a*) there is considerable condensation of moisture on the lids of the Petri dishes, and this must have been extracted from the culture medium, thus leading to a certain degree of desiccation. The latter factor in itself tends to increase sporulation of many fungi, including *B. cinerea* and *B. gladiolorum* (see below), but subsidiary experiments showed that light was the essential factor. Thus conidial formation was not materially affected by turning the dishes upside-down (which eliminates condensation on the lids), and conversely no conidia were produced in blackened Petri dishes placed with the lids uppermost, though here condensation of moisture was well marked.

Of the media tested, the glucose-peptone medium and an extract of gladiolus leaves proved to be the most suitable. Potato and malt extracts were of little value.

That a certain degree of drying accentuates spore production has been shown by Reidmeister (1909), Wade (1945) and others. The following method, which combines the effects of light, suitable medium, and a certain amount of drying, was developed and proved satisfactory for the supply of spores of *B. gladiolorum* for experimental purposes. The fungus was grown in the light on the liquid glucose-peptone medium already mentioned, and after ten to fourteen days the hyphal mats were placed on sterile filter-papers on wire gauze in large dry glass dishes. These were then kept exposed to light. Within two to three days numerous conidia developed on the slowly drying mats, especially round the edge and on the originally lower surface.

A point of interest that appeared in these experiments was that the conidia of *B. gladiolorum* germinate comparatively easily. Dishes which showed an abundance of conidia would, especially under favourable humidity, show a replacement of the conidia with white woolly mycelium within four or five days. This tendency was not apparent in *B. cinerea* where conidia persisted as such for a long time.

DESCRIPTION OF *BOTRYTIS GLADIOLORUM* TIMMERMANS, THE CAUSAL ORGANISM OF *BOTRYTIS* ROT OF GLADIOLUS

This fungus is sufficiently distinctive in its cultural and morphological features to merit specific status. In its pathogenic behaviour it stands in strong contrast to the other isolates of *Botrytis* tested in that it was the only one which actively invaded gladiolus tissues; conversely it stood out from the remainder in causing negligible attack on such hosts as turnip, swede and lettuce, on which isolates of *B. cinerea* were active.

The following description of the fungus is based on my experience, and is in substantial agreement with Timmermans's account.

Mycelium on gladiolus corms white woolly, often profuse; on artificial media similar; mature hyphae 8–12 μ diameter, finer branches down to 3 μ; in culture grey-green appressoria formed along the glass of Petri dish or tube, but this feature tends to become less prominent in long-established cultures.

Sclerotia on corms developed abundantly, first as smooth creamy white bodies soon becoming black and by-and-by usually coalescing into large coralloid masses; in artificial cultures usually occurring rarely and then in old cultures (one month old). (In contrast to this, sclerotium-forming isolates of *B. cinerea* formed sclerotia in comparatively young cultures). Sclerotia normally small in culture, but reaching 1 cm. in length on a glucose-peptone medium; in later stages of the work showing a tendency to become submerged in the medium.

Microconidia formed on most media on small side branches of the hyphae, small, globose, 2 μ in diameter.

Macroconidia freely produced on flowers in the field and to a less extent on corms; in artificial culture sparingly formed and tending to become rarer by continued subculturing; produced more freely under conditions of abundant light, etc., as outlined in the preceding section; borne on simple or branched conidiophores which are greyish brown (not dark brown as described for *B. gladioli* by Klebahn), but the precise colour is probably conditioned by the environment, as darker conidiophores have been seen in culture on glucose-peptone medium in good light; conidiophores paler towards top, average diameter up to 18 μ, cells variable in length; conidia borne on ampulles on main stalk or side branches, terminal or lateral; uniform in size, averaging $13 \times 8\,\mu$, generally 1-celled, ovoid, formed in the presence of light, germinating generally by one or sometimes two germ tubes.

In addition to the normal conidia, abnormal conidia are often seen; these arise on more hyaline conidiophores. The end of such a hypha swells to varying degrees and buds off the conidia terminally or laterally. Often the whole ampulle with its buds is detached or the end of a hypha forms a spore without budding. These spores are sometimes bicellular, but their most striking feature is their variable length, which may reach 50 μ. They may sometimes be found germinating in situ. They are generally found in cultures which have received suboptimal illumination, but may also occur mixed with conidia of the ordinary type. Similar structures were seen in a culture of *B. gladiolorum* supplied by Dr Timmermans.

After the conidia have been formed the ampulles always shrivel in the way figured by Timmermans (1942).

Average spore measurements from my data and from the literature are assembled in Table 12.

Table 12

Author	Isolate	Av. spore measurement	Quotient*
Peiris	*B. gladiolorum*	13·6 × 10·2	1·33
	B. cinerea	10·1 × 8·0	1·24
Timmermans (1942)	*B. gladiolorum*	15·0 × 10·0	1·5
Moore (1939)	*Botrytis* from gladiolus	13·0 × 10·0	1·3
Wade (1945)	*Botrytis* from gladiolus	15·0 × 12·0	1·25
Dodge & Laskaris (1941)	*Botrytis* from gladiolus	15·8 × 10·5	1·5
Klebahn (1930)	*B. gladioli*	10·4 × 4·7	2·21

* Quotient = length ÷ breadth

It is evident that with the exception of *B. gladioli*, the other isolates from gladiolus approximate to the *B. gladiolorum* of Timmermans. The spores of *B. gladioli* were measured in glycerine, and Klebahn suggests that for comparison the readings should be increased by one-third. Even after making this allowance, the average measurements for *B. gladioli* would be 13·8 × 6·3μ, the spores being characterized by their narrowness as reflected by the high quotient 2·21. The other nine species of *Botrytis* which Klebahn studied had a markedly smaller quotient. These measurements and the peculiar jagged or toothed sterigmata of *B. gladioli* have not been observed by subsequent workers, and it is unknown whether *B. gladioli* was pathogenic on gladiolus, as Klebahn did no inoculation experiments. Wade (1945) is doubtful whether his isolate, in spite of the difference in spore measurements, could be regarded as a different species from *B. gladioli*. There are, however, strong resemblances between Wade's isolate and that described by Timmermans (1942) and here as *B. gladiolorum*. The fungus described by Dodge and Laskaris (1941), though not worked out in detail, also shows similarity with *B. gladiolorum* especially in its poor sporing capacity in culture. The isolates of Moore (1939) and Hawker (1946) were recognized by them to be different from *B. gladioli*. It is possible that they were isolates of the species *B. gladiolorum*.

SUMMARY

1. The symptoms of the disease studied are in substantial agreement with those described by Moore and the three types of corm symptoms mentioned by him are different phases of the same disease.

2. The symptoms are constantly associated with a species of *Botrytis* different from *B. cinerea*. Morphological and cultural examination of this fungus shows that it is *B. gladiolorum* Timm.

3. Inoculation experiments through wounds on corms showed that *B. gladiolorum* is more pathogenic on gladiolus than *B. cinerea*. The rate of attack is greatest at a storage temperature of about 15° C.

4. On flowers and shoots *B. gladiolorum* is also more pathogenic, but although under ideal conditions foliage attack caused subsequent corm

rot, this is probably not of major importance in regard to storage rot. The evidence shows that the higher proportion of infection leading to storage rot occurs after lifting the corms, probably on the drying racks.

5. A useful measure of control of the disease is obtained by avoiding planting in heavy soil liable to waterlogging, by early lifting, by rapid drying at a high temperature, and by early cleaning.

6. *B. gladiolorum* was pathogenic on gladiolus whereas the isolates of *B. cinerea* were comparatively inert. Converse pathogenicity was observed when turnips, swedes and lettuce were used as hosts.

7. *B. gladiolorum* tends to be sterile in artificial culture. Media suitable for sporulation are a decoction of gladiolus leaves and glucose-peptone. Formation of conidia occurs only in good light and is also accentuated by moderate drying of the culture medium.

8. A description of *B. gladiolorum* is included which is in agreement with that of Timmermans. The features of this isolate are considered to be sufficiently distinctive to entitle it to specific status. The isolates of *Botrytis* from gladiolus made by earlier workers except Klebahn show in greater or less degree similarities with *B. gladiolorum* and were probably isolates of *B. gladiolorum* Timmermans.

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SPONTANEOUS MUTATION IN STANDARD AND '*GIGAS*' FORMS OF *PENICILLIUM NOTATUM* STRAIN 1249 B21

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(With Plate XXIII and 1 Text-figure)

Camphor-induced '*gigas*' forms have been produced by Bauch in yeast and other fungi (Bauch, 1941 *a*, *b*), in *Torulopsis utilis* by Thaysen and Morris (1943), in *Saccharomyces cerevisiae* (Subramaniam, 1945) and in *Penicillium* (Sansome, 1946; Kostoff, 1946). Bauch stated that natural camphor was more active than synthetic camphor. The sample of camphor used in these experiments was determined by Prof. Brindle to be dextrorotatary and it is, therefore, most probably natural camphor. Skovsted (1947), using synthetic camphor, was unable to obtain diploid from haploid strains of *Saccharomyces cerevisiae*. Kostoff obtained *gigas* forms after acenaphthene treatment as well as after treatment with camphor.

What is the nature of these *gigas* strains? Bauch assumed them to be diploids, presumably on the basis of the size difference which is of the order to be expected if the original forms are haploid and the *gigas* forms are diploid. The cytology of these forms is very difficult, and so far no convincing direct cytological evidence regarding chromosome numbers in these forms has been obtained. In *Penicillium notatum* strain 1249 B21, certain mutant types occur with a high frequency, as previously described (Sansome, 1947). It was thought that if *gigas* types of this strain could be obtained, a study of mutation in the standard and *gigas* forms might provide genetical evidence as to the nature of the *gigas* forms.

Experimental

Plates of malt-extract agar were prepared with small amounts of camphor and inoculated together with control plates without camphor. The cultures could not be placed in the incubator because of the risk of treating other cultures in the incubator with camphor vapour. They were, therefore, left in the laboratory under bell jars at a much lower temperature than that of the incubator. The necessity of growing the cultures at a much lower temperature than usual may have been an advantage, since Levan (1947) has shown that the action of camphor and other substances on the growth of yeast is intensified at lower temperatures. Camphor has a powerful inhibitory effect on the growth of *Penicillium*. The hyphae are short, much branched and twisted, forming a small colony with an irregular wavy edge. Conidial formation is much delayed and it is possible that it occurs only after the concentration of camphor has been reduced by evaporation.

When conidia appeared they were plated out by the dilution method and a number of single conidial cultures were isolated into tubes of malt-extract agar. These were later examined for conidial size. No cultures were found to have distinctly larger conidia than the controls, but there was much variation in conidial size which made the recognition of *gigas* cultures difficult. The experiment was, therefore, repeated, using glycerine-molasses-peptone agar which induces heavy conidial formation; since it was thought that this medium would also lead to the production of conidia more regular in size. This occurred, and as a result of the second experiment two out of thirty cultures were obtained, with conidia larger than those of the controls. It must also be mentioned that one out of thirty of the controls had larger conidia also. From this experiment it appears *gigas* forms may appear spontaneously in this strain when growing at rather low temperatures and it is not certain that the two *gigas* forms obtained in the treated series were actually the result of the treatment.

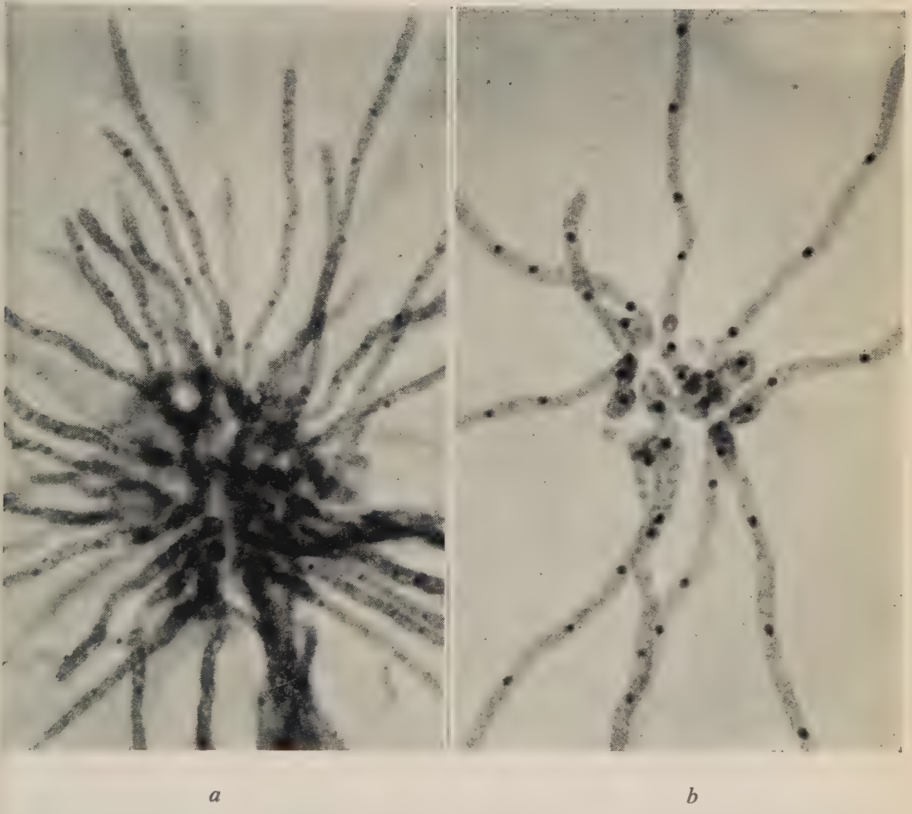
Comparison of standard and gigas *forms*

The average diameter of the conidia of the *gigas* form is $4{\cdot}2\mu \pm 0{\cdot}04$ as compared with $3{\cdot}0\mu \pm 0{\cdot}01$ for the standard. The ratio is thus 1·4:1. The probability p that the two types of conidia belong to one group is less than 0·01. The penicilli of the *gigas* form are slightly larger than those of the standard as illustrated in Text-fig. 1. There is much variability in hyphal width so that comparisons between the two types for this characteristic are difficult. Pl. XXIII shows germinating conidia of *gigas* and standard forms of strain 1249 B21 fixed in formol alcohol and stained with Heidenhain's haematoxylin. The size difference, less easy to detect in the ungerminated conidia is obvious in the swollen germinated conidia. A point of special interest is that the nuclei of the *gigas* form are much larger than those of the standard type. This is in accordance with the hypothesis that the *gigas* type is diploid although not, of course, a proof of it, since a general size factor would affect nuclear as well as cell size.

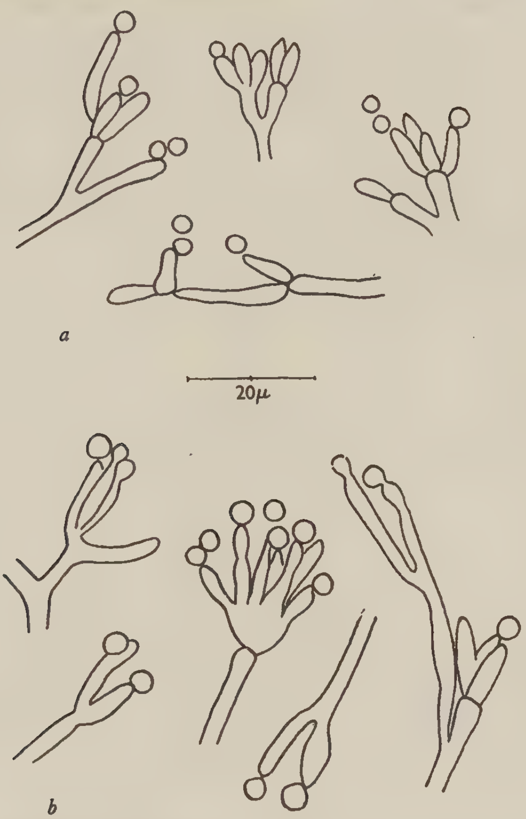
Apart from the microscopic size differences the *gigas* and standard forms are identical in appearance and when inoculated together they give a colony completely homogeneous in appearance.

Mutation in gigas *and standard forms*

Table 1 shows the spontaneous mutant colonies obtained by plating out eight single conidial cultures of the standard type. There were 0·7% of *dark* 1 variants, 0·9% of *pale* 1 variants and 0·2% of other variants. Three-quarters of the last class came from one culture and consisted of similar mutants which probably came from one original mutation. Every culture gave both *pale* 1 and *dark* 1 mutants but one, in which the *pale* 1 type was missing. In this case only 281 colonies were recorded, and it is possible that if more had been examined the *pale* 1 type would have been found here also. Of the total mutants amounting to 1·7% over 1·5% were either *pale* 1 or *dark* 1. This is in accordance with previous results, in which it was



Germinated conidia of (*a*) standard, (*b*) *gigas* strains, fixed formol-alcohol, stained with Heidenhain's haematoxylin, ×1000.



Text-fig. 1. Penicilli from (*a*) standard, (*b*) *gigas* forms. The metulae and sterigmata are longer in the *gigas*-form.

Table 1. *Spontaneous mutants in control standard*

Age of single conidial cultures (days)	Total colonies recorded	Dark 1	Dark 1 (%)	Pale 1	Pale 1 (%)	Others	Others (%)	Total	Total (%)
11	1345	6	0·45	19	1·41	1	0·17	26	1·93
11	532	9	1·69	9	1·69	—	—	18	3·38
11	1338	4	0·30	7	0·52	—	—	11	0·82
14	932	9	0·97	15	1·61	—	—	24	2·58
99	225	5	2·22	1	0·40	—	—	6	2·67
11	541	2	0·37	2	0·37	9	1·66	13	2·40
11	1247	5	0·40	3	0·24	—	—	8	0·64
14	281	4	1·42	—	—	2	0·71	6	2·14
Total	6441	44	0·7	56	0·9	12	0·19	112	1·74

shown that the greatest part of the variability of strain 1249 B21 is due to the occurrence of these two types of mutants (Sansome, 1947).

Table 2 shows the spontaneous mutants from thirteen single conidial cultures of the *gigas* form. The cultures marked with an asterisk had small darker sectors. The remainder were uniform in macroscopic appearance. Every culture gave a certain number of paler and darker variants. The total number of variant colonies was 226 out of 4872 or 4·6%; excluding the sectored cultures the variants were 4·3%. The darker variants amounted to 2·5% and the paler variants to 1·1% when the sectored cultures were included. When the sectored cultures were excluded the darker variants amounted to 2·0% and the paler variants to 1·2%. Spasmodically occurring mutants of different types amounted to 1·1% of the total and were thus more frequent in the *gigas* form than in the standard, where they were only 0·2% of the total colonies.

Table 2. *Spontaneous mutants in standard* gigas *form*

Age of single conidial cultures (days)	Total colonies recorded	Dark variants	Dark variants (%)	Pale variants	Pale variants (%)	Other variants	Other variants (%)	Total variants	Total variants (%)
11	592	6	1·01	3	0·51	—	—	9	1·52
11	631	14	2·22	2	0·32	—	—	16	2·54
11	240	6	2·50	5	2·08	1	0·42	12	5·00
11	273	2	0·73	4	1·47	1	0·37	7	2·56
11	412	6	1·46	2	0·49	5	1·21	13	3·16
11	185	1	0·54	1	0·54	—	—	2	1·08
14	353	7	1·98	4	1·13	3+7 sect.	2·83	21	5·95
	238	3	1·26	3	1·26	—	—	6	2·52
41	271	16	5·90	6	2·21	9	3·32	31	11·44
42	476	11	2·31	13	2·73	18	3·78	42	8·82
11*	372	23	6·18	2	0·54	1	0·27	26	6·99
11*	387	20	5·17	5	1·29	4	1·03	29	7·49
11	442	7	1·58	2	0·45	3	0·68	12	2·71
Total	4872	122	2·5	52	1·1	45+7 sect.	1·1	226	4·6
Excluding sectored cultures	3671	72	2·0	43	1·2	37+7 sect.	1·2	1·159	4·33

The darker and paler types were more difficult to record in the *gigas* form than in the standard, being intermediate between standard and *dark* 1 and standard and *pale* 1 respectively. However, with practice it was possible to record the darker variants with reasonable accuracy. The recording of the pale types was more difficult and subject to greater error. A number of the dark variants coming from different cultures were inoculated together. They gave apparently homogeneous colonies.

Since the dark and pale variants in the *gigas* form were intermediate in appearance between the standard type and *dark* 1 and *pale* 1 respectively, it was thought that they might be heterozygotes for the *dark* 1 and *pale* 1 factors, one chromosome being changed, the other not. If so, the intermediate types might be expected to give mutants more closely resembling the mutants from the original standard type by the mutation of the second chromosome. The mutants would be expected to occur about as often as in the original standard type. The intermediate dark type was chosen for more detailed study rather than the intermediate pale for two reasons. In the first place, the intermediate darks were more easy to record;

secondly, the original dark variant was relatively stable whereas the *pale* 1 type was unstable. Consequently, the mutational pattern of the intermediate dark type might be expected to be simpler than that of the intermediate pale type. One or two of the intermediate pale cultures were plated out and they gave a range of paler variants in accordance with this expectation.

Table 3. *Spontaneous mutants in intermediate dark* gigas *form*

Age of single conidial cultures (days)	Total	Dark mutants	Dark mutants (%)	Paler mutants	Paler mutants (%)	Other mutants	Other mutants (%)	Total mutants	Total mutants (%)
14	1032	8	0·78	33	3·20	5	0·48 sect.	46	4·46
12	801	9	1·12	—	—	11	1·37 sect.	20	2·49
12	224	13	5·80	3	1·34	2	0·89	18	8·04
12	936	8	0·86	11	1·18	23	2·46 sect.	42	4·49
12	360	6	1·67	1	0·28	3	0·83	10	2·78
40	338	8	2·37	9	2·66	4	1·18	21	6·21
Total	3691	52	1·41	57	1·54	9+39 sect.	1·30	157	4·25

Table 3 gives the spontaneous variants from six single conidial cultures of the *gigas* intermediate dark type. Each culture gave a number of darker types, corresponding in appearance with the *dark* 1 type from the original 1249 B21. When such dark types of different origin were inoculated side-by-side they gave apparently homogeneous colonies. Similarly, when this *gigas* 2-step dark was inoculated with a standard 1-step dark an apparently homogeneous colony resulted. So far as may be tested, therefore, the *gigas* form gives, in two steps, the mutant given in one step by the standard form, as expected if the *gigas* form is diploid and the intermediate type is the heterozygote. This expectation is dependent upon the mutation being of nuclear rather than cytoplasmic origin.

The percentage of 2-step darks was 1·4, rather more than the 0·7% of *dark* 1 mutants produced by the standard and less than the 2·0% of intermediate dark mutants produced by the *gigas* type. These percentages are in the relationship to be expected, but too much weight must not be attached to this because of the great variance.

A point of interest is that although the original *dark* 1 type was very stable, giving only rare *dark* 2 variants, and never reverting to the parent type, the intermediate *gigas* darks gave some paler types. Some of these paler types were identical with the original *gigas* type so far as could be tested.

The exceptional 'intermediate dark' from the standard 1249 *B*21

In one of the control cultures plated at the same time as the *gigas* intermediate darks a variant strongly resembling the intermediate dark type appeared along with the usual *dark* 1 type. The possibilities that the control might have been a spontaneous *gigas* form or that the intermediate darks might be contaminants from the *gigas* cultures, were eliminated by an examination of the conidial size. The exceptional variant and its sister colonies were found to have conidia of the standard size.

A number of single conidial cultures of this intermediate dark type were obtained and plated out. Table 4 gives the spontaneous mutants obtained from five of these cultures. There were 0·3% of darker mutants and 0·7% of paler mutants. However, these mutants were quite unlike those given by the *gigas* intermediate dark type, the dark types being much darker and the pale types much paler. Although this exceptional dark type closely resembles the *gigas* 1-step dark on the basis of our first criterion, appearance, it has a very different mutational pattern and must, therefore, be assumed to be genetically distinct. The simplest explanation is that it represents a different mutation which intensifies the effects of the *pale* 1 and *dark* 1 mutations.

Table 4. *Spontaneous mutants in intermediate dark from control*

Age of single conidial cultures (days)	Total colonies	Darker mutants	Darker mutants (%)	Paler mutants	Paler mutants (%)	Other mutants	Other mutants (%)	Total mutants	Total mutants (%)
15	490	—	—	—	—	2 sect.	0·41	2	0·41
28	580	2	0·34	3	0·52	—	—	5	0·86
21	159	2	1·26	2	1·26	—	—	4	2·52
21	357	1	0·28	5	1·40	—	—	6	1·68
21	206	1	0·49	3	1·46	—	—	4	1·94
Total	1792	6	0·33	13	0·72	2	0·11	21	1·17

DISCUSSION

Gigas forms of 1249 B21 have been shown to give a mutant type identical in appearance with the *dark* 1 type given by the parent form. However, they give the mutant in two steps, the first change being to a type intermediate between the standard and *dark* 1 types. These results are readily explicable if the original type is haploid and the *gigas* form diploid. In that case the intermediate dark would be the heterozygous dark formed by mutation of one chromosome to the *dark* 1 type. Mutation of the unchanged chromosome of the heterozygous type would give the type identical in appearance with the *dark* 1 of the original form.

If the proportion of nuclei mutating to *dark* 1 in the haploid is x, the proportion mutating to the intermediate dark type in the diploid would be $2x - x^2$, and the proportion of nuclei mutating directly to the *dark* 1 type would be x^2. Since x^2 would be a small quantity, there should be almost twice as many nuclei mutating to the intermediate dark type in the diploid as to the *dark* 1 type in the haploid. Actually, there were 0·7% of *dark* 1 types in the control cultures and 2% of darker mutant colonies in the *gigas* cultures (excluding those cultures with a visible mutant sector). This is in accordance with expectation but the difference is not significant because of the great variance. Also, it must be emphasized that the proportion of mutants recovered depends upon other factors than the mutation rate: such as time of occurrence of mutations, relative survival values of original and mutated types and degree of conidial formation of the two types. Furthermore, the Sewall-Wright effect which acts in favour of the nuclei present in the greatest numbers would be less strongly in favour of the original type when the number of mutations is increased. This probably accounts for the increase in colonies with visible mutant sectors in the *gigas* form.

The change from intermediate dark to the 2-step dark, since it involves the mutation of the one unchanged chromosome would be expected to occur at about the same rate as the change from standard to *dark* 1 in the controls. Actually, the controls gave 0·7% of *dark* 1 variants whereas the *gigas* intermediate darks gave 1·4% of the 2-step type. However, there is another phenomenon to be considered here. The *dark* 1 type given by the controls has never been observed to revert to the standard type. On the other hand, the *gigas* intermediate dark type frequently gave a paler variant, indistinguishable from the *gigas* standard type in appearance and having the same mutational pattern. It is probable that this type results from somatic crossing over in the intermediate dark type. If this is so, a certain proportion of the 2-step darks should also result from crossing over rather than mutation. In a nucleus heterozygous for the *dark* 1 mutation, crossing over between the standard chromosome and the mutated chromosome would give two daughter nuclei, one homozygous for the mutated chromosome, the other homozygous for the standard chromosome. Thus if the reversions to the standard type are due to somatic crossing over there should be a corresponding increase in the 2-step dark types. Since it was not always possible to distinguish between the paler variants corresponding to the standard type and other pale variants given by the intermediate *dark* type, they were recorded together in Table 3, and it is not possible to say how much of the increase over expectation in the 2-step dark class could be accounted for on the basis of somatic crossing over.

The frequencies of spasmodic mutants, that is those not included in the mutable series in the standard and *gigas* types are of interest. There were 0·2% of these in the controls, and 1·1 and 1·3% in the *gigas* forms. The occurrence of mutants is thus much more frequent in the *gigas* forms; it might have been expected that the *gigas* type would be less mutable than the original type on the hypothesis that the original type is haploid and the *gigas* type diploid, since mutations in one chromosome would be covered by the normal allele in the other chromosome. However, *Penicillium* normally exists in the haploid condition, and if dominance of the wild type is largely the result of natural selection, as suggested by Fisher, there is no mechanism by which such dominance could be evolved in *Penicillium*, comparable to that of diploids or of heterokaryotic haploids. In this connexion, it may be mentioned that the supposed heterozygote for the standard and *dark* 1 type is intermediate, neither type being dominant. The residual mutants in the *gigas* form are mostly of reduced vigour as compared with the standard type and it is probable that they represent mutants which are lethal in the haploid and would be lethal when homozygous in the diploid.

The action of camphor on the nuclei of plants where chromosome counts can be made without ambiguity may be considered in relation to the nature of the *gigas* forms induced by camphor treatment in yeast and other fungi. Camphor has been shown to have an effect on mitosis in onion root tips similar to the effect of colchicine (Ostergren & Levan, 1943). This has been called the *c*-mitotic effect (Levan, 1938). Levan (1947) and Levan & Sandwall (1943) have made an extensive study of the effect of camphor and other substances on the growth of yeast cells and have observed the formation of polyploid nuclei in treated material. Colchicine, the agent which is generally most efficient in inducing polyploidy in higher organisms, is ineffective on the fungi and in particular on *Penicillium* (Sansome & Bannan, 1946). The action of colchicine in inducing polyploidy is primarily due to its effect on the spindle. Since fungi have intranuclear spindles as contrasted with the extranuclear spindles of higher plants, it might be thought that the ineffectiveness of colchicine is due to the difference in spindle formation. Then one would expect that other polyploidy-inducing substances might be similarly ineffective when applied to fungi. However, *c*-mitotic substances have other effects besides their effect on the spindles as discussed in detail by Ostergren (1944). These effects include the *c*-tumour effect and an effect on chromosome contraction. When *Penicillium* and other fungi are treated with colchicine none of these effects can be recognized; the fungal growth is apparently normal. Therefore it would seem that the ineffectiveness of colchicine in inducing polyploidy in fungi is due to a general resistance to the effects of the drug rather than to the method of spindle formation. When *Penicillium* is subjected to camphor treatment, the growth is greatly affected, the hyphae are distorted, short, much branched and thickened. This effect on growth may be analogous with the *c*-tumour effect of colchicine and other substances, including camphor, on higher plants. Camphor has also a pronounced effect upon the growth of yeast as studied particularly by Levan. Cell formation is interfered with so that multinucleate hyphal-like cells are formed, and there is a tendency for the cells to stick together and form colonies.

Skovsted (1947) treated haploid cells of *Saccharomyces cerevisiae* with solutions of synthetic camphor and failed to get diploid forms, although he obtained more mutants from treated than untreated material. Bauch obtained his *gigas* forms by using natural not synthetic camphor, and Skovsted's failure to obtain diploids may be due to the fact that he used synthetic camphor. If the dextrorotatary form of camphor is more efficient than the laevorotatary form and if both forms are equally lethal, when synthetic camphor which is composed of a mixture of these two forms is used, it may be that the threshold for induction of *gigas* forms is so near that for lethality that the probability of obtaining *gigas* forms is greatly reduced. Both forms of camphor might be equally effective in producing the characteristic camphor growth forms.

Thaysen and Morris (1943) obtained *gigas* forms after camphor treatment in *Torulopsis utilis*, a non-spore-forming yeast which is probably haploid. In this fungus *gigas* forms only resulted from the treatment and there was no necessity to isolate the *gigas* from the original form. It would seem that the *gigas* forms are more resistant to the effect of the camphor since they are able to survive doses which are lethal to the original type. In this experiment the possibility that the camphor treatment selects spontaneously occurring large mutant types has not been eliminated.

It has been suggested that some of the *gigas* forms of yeast resulting from camphor treatment may owe their increased size to a cytoplasmic effect (Levan & Ostergren, 1943; Thomas, P. T., unpublished). Levan & Ostergren suggest that dauermodifications produced by *c*-mitotic substances may be the result of a change in some lipoid-containing cytoplasmic units such as mitochondria. The hypothesis put forward by Thomas depends upon the fact that camphor treatment results in the formation at multinucleate cells. These bud off uninucleate daughter cells. It is suggested that these daughter cells of multinucleate cells are of the *gigas* type, the larger cell size being due to an effect of the cytoplasm, resulting from its having been derived from a multinucleate cell. Such an effect is supposed to persist for a time, gradually decreasing and ultimately disappearing as in the case of dauermodification.

It is difficult to see how such an explanation could hold in the case of *Penicillium* where the conidia are normally produced from multinucleate cells.

The evidence in favour of the *gigas* forms of *Penicillium* being diploid may be summarized as follows: (1) The treatment by which they have been produced has been shown to result in chromosome doubling in yeast and higher plants. (2) The difference in conidial diameter is of the order to be expected if the original type is haploid and the *gigas* type diploid. (3) The nuclei of the *gigas* type are larger than those of the original type. (4) The observations described in this paper by which the *gigas* type was found to give in two steps the change given in one step by the original type is strong genetical evidence in favour of the *gigas* type being diploid. It seems, therefore, that the *gigas* forms in *Penicillium* are almost certainly diploid and this would tend to indicate that the *gigas* forms obtained in the same way in other fungi may also be diploid. Reversion of such *gigas* forms to the original type by no means disproves the hypothesis of diploidy since occasional reduction divisions might occur and if the original form has a higher growth rate than the *gigas* form such occasional reduction divisions could result in reversion of the cultures to the original type. The difference between this type of reversion and that expected on the hypothesis of cytoplasmic effect is that the latter type would be gradual and the former abrupt. Some of the large-celled types observed in yeast which have reverted comparatively soon to the original type may well be due to cytoplasmic effects. Any such temporary effects would not have been observed in these experiments on *Penicillium* because the method of isolating *gigas* forms would tend to eliminate changes of a temporary character.

SUMMARY

Spontaneous mutation was studied in standard and *gigas* forms of strain 1249 B21.

The standard regularly gave a darker variant *dark* 1. The *gigas* form similarly gave a darker variant type which was intermediate in appearance between the standard and *dark* 1 types. This intermediate dark type gave variants closely resembling the *dark* 1 type.

The proportions in which these types were recovered are consistent with the hypothesis that the *gigas* form is diploid and that its intermediate dark variants are heterozygous for the *dark* 1 type.

The nature of the *gigas* forms is discussed in relationship to these results and to the observations of other works on yeast. It is concluded that they are probably diploids.

Thanks are due to Imperial Chemical Industries, Ltd. for financial support, and to Prof. C. W. Wardlaw for constant help and encouragement.

The text photographs were taken by Mr E. Ashby.

A Kodachrome transparency showing the differences between the standard, the intermediate dark and the *dark* 1 types, and also the similarity between the *dark* 1 type and the *gigas* 2-step dark has been obtained. It was hoped to produce a colour plate from this but the chance of losing these differences on reproduction is considered too great to warrant the expense of a colour plate. The original Kodachrome taken by Mr E. Young, will be deposited in the department of Cryptogamic Botany of Manchester University.

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THE DEVELOPMENT OF FRUCTIFICATIONS OF *LENTINUS TUBER-REGIUM* FRIES, IN CULTURE

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(With Plate XXIV and 7 Text-figures)

In the Belgian Congo, Prof. C. W. Wardlaw found specimens of *Lentinus tuber-regium* Fries, growing among the leguminous cover crops which form part of the ground vegetation of Oil Palm plantations. This fungus produces very large spherical sclerotia which, as described by Engler and Prantl (1900), and as observed by Prof. Wardlaw, may be as large as 10 in. in diameter. Fructifications normally develop from these sclerotia (Pl. XXIV, fig. 1). One of the sclerotia was cut open, and fungal material taken from the medium-soft pseudo-tissue and transferred to 3% malt agar slopes. These cultures were brought back to Manchester and used in the work to be described.

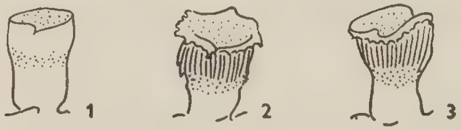
Transfers were made to various agar culture media and to different types of soils and decaying organic materials in flasks. These cultures were incubated at 24° C. in the dark. A white, fluffy mycelium grew vigorously over the surface of the agar media and spread throughout the soils, but fructifications developed only on potato agar and on riddled loam (with or without the addition of chopped leaves and stems of leguminous plants).

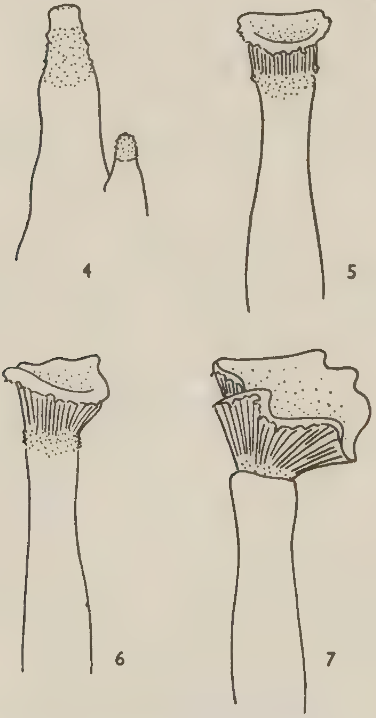
After three weeks' growth on potato agar, small, dark, rounded papillae, 5–10 mm. in diameter, formed in the centre of the cultures and after a further five days in the light at laboratory temperature, one of them developed into a cylindrical structure with a concave, scaly upper surface and a spotted region round the middle (Text-fig. 1). The following day, gills developed in the pale region at the top of the vertical sides of the fructification (Pl. XXIV, fig. 2; Text-figs. 2 and 3).

On the loam soils, after five to six weeks, several small, white, solid papillae formed round the sides of the flasks and after a further three weeks developed into long cylindrical rods, about 5 mm. in diameter, which grew upwards from between the soil and the sides of the flasks (Pl. XXIV, fig. 3). No further development took place in the dark. When the cultures were brought into the light, one rod in each flask became flattened on top and speckled (Text-fig. 4; Pl. XXIV, fig. 4), and after four days, a pileus with gills developed terminally on the rod which thus became the stipe (Pl. XXIV, fig. 5; Text-figs. 5–7). The pilei were funnel-shaped, pale-coloured and had dark brown scales. The edges were wavy and broken and the lamellae were decurrent, pale-coloured at first, becoming light brown. The upper parts of the long, slender stipes were also scaly.

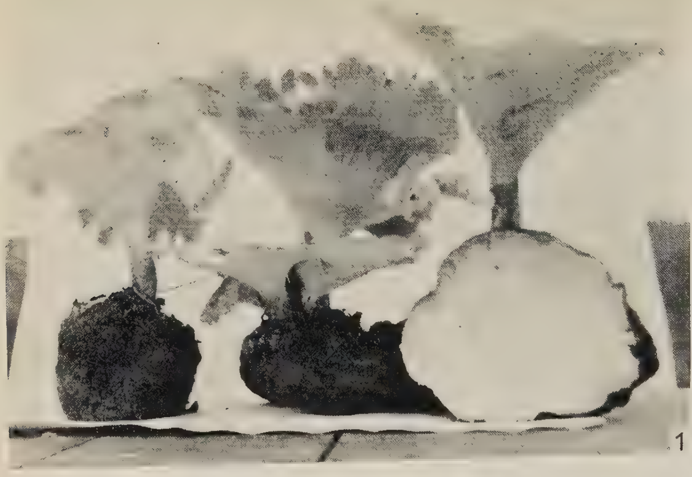
Thus, fructifications of *Lentinus tuber-regium* were produced from mycelial cultures without the development of the large sclerotia on which the fructifications were growing under natural conditions.

Light apparently has a formative effect on the development of the pileus of *Lentinus tuber-regium*, since in the dark the cylindrical rods continue to grow in length but do not produce pilei. Buller (1905) described the same phenomenon for *Lentinus lepideus* and suggested that sensitivity to light might be a feature of agarics which do not grow on the ground. He stated that *Agaricus campestris*, a ground agaric, usually has a substratum with a horizontal surface, and once a fruit body has begun to form on the surface of the ground, the stimulus of gravity alone is sufficient to bring about the development of the stipe and pileus in vertical sequence with the subsequent uplifting of the latter into the air. The orientation of the surface of the substratum of *Lentinus lepideus*, a tree agaric, is, however, indefinite but response to light causes young fruit bodies to grow outwards through cracks in the bark, with the subsequent development of the pileus in the stronger light of an open space.





Text-figs. 1–3. Drawings showing stages in fructification formation on potato agar.
Text-figs. 4–7. Drawings showing stages in fructification formation on loam soil.



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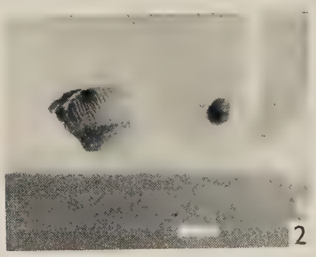

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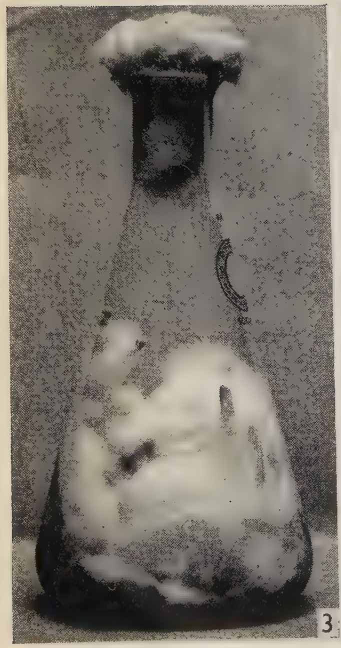

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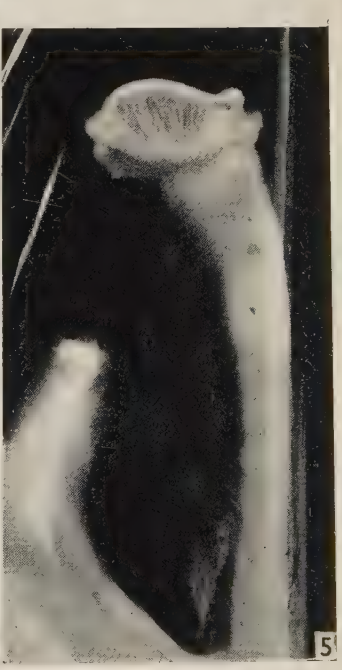

5

The reaction of *Lentinus tuber-regium* to light is not in agreement with Buller's hypothesis that all ground agarics would behave like *Agaricus campestris* with respect to the effect of light, since it, a ground agaric, is dependent on light for the formation of a pileus. A modification of Buller's hypothesis is, therefore, necessary in view of the results of this experiment.

SUMMARY

1. Fructifications of *Lentinus tuber-regium* normally develop on the large sclerotia which are to be found among the ground vegetation of Oil Palm plantations in the Belgian Congo.

2. A description is given of the development of fructifications from mycelial cultures without the formation of sclerotia.

3. The formative effect of light is discussed.

In conclusion I wish to thank Prof. C. W. Wardlaw and Dr A. R. Gemmell for their help and criticism, and Mr E. Ashby for his preparation of the photographs.

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EXPLANATION OF PLATE XXIV

Fig. 1. Sclerotia and fructifications of *Lentinus tuber-regium* as found on Oil Palm plantations in the Belgian Congo. (× about $\frac{1}{7}$.)

Fig. 2. Fructification grown on potato agar. (Nat. size.)

Figs. 3–5. Stages in fructification formation on loam soil. (Fig. 3 × about $\frac{1}{3}$. Figs. 4 and 5 nat. size.)

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LIST OF FUNGI RECORDED AS PATHOGENIC FOR MAN AND HIGHER ANIMALS IN BRITAIN

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Introduction

The census of fungi recorded for Britain taken under the Society's auspices did not include 'medical fungi' because the records for these forms were widely scattered in books and journals not easily accessible to the members who undertook the preliminary search of the literature. The present list, which is an attempt to fill this gap, differs from others in the series in several ways. It is, being primarily a catalogue of pathogenic species, not confined to one taxonomic group and it includes certain actinomycetes which tend to be referred to the mycologist rather than the bacteriologist. The search of the literature (to the end of 1947) has been less thorough. Also, as medical mycologists have nothing to correspond with the *List of Common British Plant Diseases*, disease names have, whenever possible, been equated with those of important pathogens.

Study of the literature raised many problems of selection. Uncertainty as to the identity of the fungi involved necessitated the exclusion of much material. More than seventy-five papers on the dermatophytes and dermatomycoses were excluded for this reason. Uncertainty as to the pathogenicity of some of the fungi reported to have been associated with disease in man and animals led to the rejection of other records. Obvious saprophytes have been omitted and certain species included have been listed as 'isolated from' some particular organ instead of 'on' man or other animal when their status as pathogens appeared doubtful. A few poisonous fungi have also been noted. Another difficulty was to find records for even some of the common pathogens which were satisfactory from a mycological point of view. The published account of a case studied in this country has been treated as a British record even if the infection was contracted abroad. An effort has, however, been made to distinguish exotic diseases (such as mycetoma and rhinosporidiosis) from endemic diseases. Studies made in this country on non-British material have been included only when there has been a British case of the particular disease.

Most of the many text-books of bacteriology and dermatology mention fungi causing mycoses, and reference has been made to a few representative examples. These are mostly works by medical men known to have had an interest in pathogenic fungi, and reference is usually made to the latest editions which may be taken as representing the authors' considered opinions. Important clinical papers have also been compiled because clinical data cannot be ignored by the mycologist. Published notes on cases exhibited to the various medical societies have, however, been excluded unless they are first records for the country or provide other points of particular interest.

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Attention is drawn to the fact that this list, like its companions, is not based on any extensive examination of specimens and the synonymy adopted will no doubt need to be modified. For example, the organisms causing actinomycosis in man and cattle are possibly distinct. Here they have been united under *Actinomyces israeli*. On the other hand, certain species of *Candida* which should probably be included in *C. albicans* have been treated as separate entities because they have been so distinguished in the British literature. The synonymies given for some species only include names which have been commonly used in this country. The index should be consulted for the formal author citations of the names of species mentioned incidentally in the body of the list.

ACKNOWLEDGEMENTS

Much of the work of compiling this list was done in the libraries of the London School of Hygiene and Tropical Medicine, the Royal Society of Medicine, and the Wellcome Historical Medical Museum. I gratefully acknowledge all that those in charge of these libraries did to lighten the task. I am also indebted to Dr J. T. Duncan and Miss E. M. Wakefield for help and advice, and to Dr P. H. Gregory for the gift of a card index to literature of the dermatophytes.

FUNGI (EUMYCETES)

PHYCOMYCETES

Absidia corymbifera (Cohn) Sacc. & Trotter. Isolated from human sputum and nasal discharge of horses, **204** (N.S. XI, 387); from milk, **70** (N.S. XIX, 314*); **146**, 23.

Coccidioides immitis Rixf. & Gilchr. Coccidioidomycosis. On man (accidental pulmonary infection in a laboratory worker), Nabarro, **140** (*1948* I, 982–4*).

Ichthyosporidium hoferi (Plehn & Muslow) Pettit. On mackerel (esp. kidney and spleen), Sproston, **169** (XXVI, 72–98*, 1944).

Rhinosporidium seeberi (Wernicke) Seeber. Rhinosporidiosis. On man (nasal polyp in an Indian medical student at Edinburgh, 1917–21), Ashworth, **53** (LIII, 301–42*, 1923); **171** (N.S. XXX, 337–51*) (reprinted **172** (XXXVIII, 285–99*)); **147**, 897*. *R. seeberi* was considered by Ashworth to have chytrid affinities but this is doubtful.

Saprolegnia ferax (Gruith) Thuret, and **S. monoica** Pringsh. Salmon disease. On salmon and other fish: W. G. Smith, **31** (IX, 560–2*, 1878); Huxley, **67** (XXXIII, 381–9, 1882); **68A** (N.S. XXII, 311–33, 1882, *S. monoica*); **168** (IX, 726–32); **27** (XXIII, 302–8), experimental inoculation of dace and salmon; Patterson, **199***, *Bacillus salmonis pestis* in relation to; **219**, 1152–60. The parasite on fish and fish eggs is considered to be distinct from *S. ferax* and *S. monoica* by Coker who named it *S. parasitica* (see Kanouse, **100** (XXIV, 431–52, 1932)).

ASCOMYCETES

Chaetomium elatum Kunze & Schmidt. Odour of, experimentally inducing nausea and giddiness in man, Blackley, **213**, 58.

Claviceps purpurea (Fr.) Tul. Ergotism. Poisoning man, **142**, 885, 887; outbreak among Jews in Manchester, **150** (*1928* I, 302), **75** (XXIX, 51, 1929); **210**.

Gibberella zeae (Schw.) Petch. On barley poisoning pigs, **22** (Bull. No. 79, 18, 1934), as *G. saubinetii*.

BASIDIOMYCETES

USTILAGINALES

Tilletia caries (DC.) Tul. Spores associated with 'epileptiform convulsions' in the dog, Greig, **191** (LXXVIII, 387, 1922), **28** (X, 121), as *T. tritici*, but in feeding experiments, spores non-toxic to dogs, rabbits, and guinea-pigs, **28** (XI, 82).

AGARICALES

Amanita phalloides (Fr.) Secr. Poisoning man. Fatalities, **140** (*1879* II, 941; *1886* II, 1018); **150** (*1905* II, 541; *1946* I, 45); **28** (III, 25, 1908). Treatment, **150** (*1947* II, 348, 395). **212**, 23; **209**, 27*; **208**, 16*.

Inocybe fastigiata (Schaeff. ex Fr.) Quél. Poisoning man, Wilson, **150** (*1947* II, 297).

I. incarnata Bres. Poisoning man, Young, **211** (XLIV, 52, 1925).

I. patouillardii Bres. Poisoning man. Fatality in Surrey, 1937 (**208**, 28*); **209**, 33.

FUNGI IMPERFECTI

MONILIALES

PSEUDOSACCHAROMYCETACEAE

Candida albicans (Robin) Berkh. Moniliasis. (Syn. *Oidium albicans*, *Monilia albicans*, *Oospora albicans*, *Monilia pinoyi*; see also *Candida metalondinensis* and *C. tumefaciens* below.) On man: buccal cavity (thrush): **181**, 63*; **161** (XXI, 221–3, 1909); **176** (CLXVII, 417, 1923); **140** (*1925* II, 1226–8); **150** (*1930* II, 119); **161** (XLIII, 354, 1931); **147**, 874*; Ludlam & Henderson, **140** (*1942* I, 64–70), neonatal thrush; **161** (LVII, 18–19, 1945); **150** (*1945* II, 716); sputum and associated with pulmonary disorders: **140** (*1923* II, 109, 110); **140** (*1925* II, 1226–8); **204** (N.S. IX, 365, 1936); Shrewsbury, **203** (XXIX (N.S., V), 375–97, 1936), secondary thrush of the bronchi, *C. albicans* not considered to be a cause of primary pulmonary disease; **150** (*1945* II, 715); **171** (LIII, 585; LIV, 421, 1946–7); skin: **140** (*1925* II, 1226–8); **161** (XXXIX, 410, 1927); **150** (*1929* II, 948); **161** (XLII, 551–9*, 1930); **157**, 1309–17*; **150** (*1945* II, 716); nail fold (paronychia): **140** (*1924* I, 128); **161** (XXXIX, 394–8, 1927; XLII, 555*, 1930; XLIII, 140–1, 1931); nails: **161** (XXI, 221–3, 1909; XXXIX, 119–22, 1927; XLII, 557*, 1930); **150** (*1930* II, 1120); **157**, 1315*; **161** (XLIII, 355, 1931); ear: **150** (*1945* II, 716); vagina: **162** (XXXII, 358, 1929); **206**, 139; **171** (XLVII, 369*, 1940);

pruritus vulvae: **162** (XXVIII, 245, 331, 1925); **189** (CXVII, 354, 1926), **161** (XLII, 559, 1930); pruritus ani: **162** (XXVII, 304–5, 1924; XXVIII, 329–31, 1925; XLI, 378, 1938). Cultural and taxonomic studies, **161** (XLII, 550, 1930); **161** (XLIV, 435, 1932), Shrewsbury, **153** (XXXVIII, 315, 1934); **161** (XLIII, 562–8, 1930), comparison with *Monilia pinoyi*; **162** (XL, 298, 300, 1937); **153** (XXXIV, 429–36, 1931), serology; **140** (*1946* I, 44–6), effect of antibiotics on; **161** (XLII, 562–8, 1930), pathogenicity to man and animals; **146**, 30; **158**, 362, 366*, 384*; **207**, 91. On birds: **174**, 140 (thrush in chickens); **175**, 457 (in poultry and pigeons).

C. guillermondi (Cast.) Langeron & Guerra. (Syn. *Endomyces guillermondi* Cast., **150** (*1912* II, 1210), *Monilia guillermondi* (Cast.) Cast. & Chalm., **205** (ed. 2, 826, 1913).) On man causing bronchomonilasis; Jockes & Simpson, **140** (*1923* II, 110). Cultural characters, **162** (XXIII, 21, 1920; XL, 301, 1937); **207**, 246.

C. macroglossiae (Cast.) Castellani & Jocono, **162** (XXXVI, 314, 1933). Isolated from case of macroglossia in man by Castellani, **162** (XXVIII, 218 (as *Cryptococcus macroglossiae*), 220* (as *Monilia macroglossiae*), 1925); **206**, 21; **144** (XXI, Sect. Trop. Dis. Parasitol. 3, 1928); Castellani & Jocono, **162** (XXXVI, 314*, 1933), as *Torulopsis macroglossiae*; Castellani, **162** (XL, 306, 1937), as *Monilia zeylanoides* var. *macroglossiae*. [= *C. zeylanoides* vide **207**, 298.]

C. metalondinensis (Cast.) Berkhout. (Syn. *Monilia metalondinensis* Cast. in **205** (ed. 3, 1082, 1919, as 'Castellani 1916'.)) On man: tonsils, Castellani, Douglas & Thompson, **162** (XXVI, 23, 1923), **206**, 113, 115, **189** (CXXIV, 74*, 1930), **144** (XXI, Sect. Trop. Dis. Parasitol. 449, 1928; and nose); vagina, **162** (XXXII, 358, 1929), **206**, 139; sputum, **206**, 123, **162** (XXXII, 5, 1929); **153** (XXXIV, 433, 1931), serology; **162** (XXIII, 21, 1920; XL, 300, 1937), cultural characters; **206**, 123, pathogenicity for rabbit. [= *C. albicans* vide **207**, 93.]

C. tropicalis (Cast.) Basgal. (Syn. *Oidium tropicale* Castellani, **150** (*1910* II, 869); *Endomyces tropicalis* (Cast.) Cast.; *Monilia tropicalis* (Cast.) Castellani & Chalmers, **205** (ed. 2, 824, 1913, as 'Castellani 1909').) On man causing thrush, Craik, **176** (CLXVII, 417, 1923, as *M. candida*); vaginitis, **206**, 139, **162** (XXXII, 358, 1929); dermal moniliasis, **150** (*1945* II, 716); in sputum, **162** (XXXVIII, 244–5, 1935), **150** (*1945* II, 715). Cultural characters, **162** (XXIII, 21, 1920; XXIV, 150*, 1921; XXVIII, 258*, 1925), **153** (XXXVIII, 315*, 1934), **162** (XL, 299*, 1937); serology, **153** (XXXIV, 433, 1931).

C. tumefaciens (Foulerton) C. W. Dodge. Isolated from two cases of pharangitis (associated with *Corynebacterium diphtheriae*), Foulerton, **153** (VI, 49, 1900), as *Saccharomyces tumefaciens albus*; **143** (I, 351, 1899), pathogenic for the rabbit. [= *C. albicans* vide **207**, 91.]

C. zeylanoides (Cast.) Langeron & Guerra. (Syn. *Monilia zeylanoides* Castellani, **162** (XXIII, 17, 1920, as 'Cast. 1917' on p. 21); **162** (XXVIII, 257–8, 1925).) On man (moniliasis of toes), Castellani, **162** (XLII, 293–5*, 1939; XLIII, 81, 1940). Cultural characters, **162** (XL, 306, 1937), **207**, 298. See also *C. macroglossiae*.

Cryptococcus farciminosus Rivolta. [*Histoplasma farciminosum*] Epizootic lymphangitis. On the horse. Introduced into this country after the South African War, last cases destroyed 1906, Pallin, **167**; **217**, 54; **219**, 1289–1301; **151**, 407–9*. Scheduled in Great Britain under the Diseases of Animals Acts.

C. gracilioides Castellani, **162** (XXVIII, 222, 1925). On man causing stomatitis (associated with *Bacillus vermiculoides* Cast.); **162** (XXIX, 224, 1926); **206**, 21, 110. [=*Pityrosporum ovale* vide Ota & Huang, *Ann. parasitol. hum. comp.* XI, 49, 1933.]

Malassezia furfur (Robin) Baillon. Tinea versicolor. (Syn. *Microsporon furfur.*) On man: **181**, 57*; **182**, 158; **214**, 463; **156**, 70–1*; **150** (*1929* II, 950); **161** (XLII, 559–61, 1930); **157**, 440–3*; **147**, 884*; **158**, 363, 388; **159**, 405–7*.

Monilia batrachea Scott, **154** (*1926*, 692), on bull frogs (*Rana catesbiana*), black spotted toad (*Bufo melanostictus*), and other amphibians; **154** (*1928*, 119), on tigrine frog (kidney, liver, and lungs).

M. londinensis Cast. in **205** (ed. 3, 1082, 1084, 1919, as 'Castellani 1916'), isolated from case of thrush in man; **162** (XXIII, 21).

Pityrosporum ovale (Bizz.) Cast. & Chalm. in **205** (ed. 2, 832, 1913, as 'Bizzozzero 1882'). On man (associated with dandruff), **156**, 72–6*, **150** (*1929* II, 950), in relation to seborrhoeic dermatitis; **161** (XLII, 559, 1930); **150** (*1930* II, 1121); **161** (XLIX, 74, 1937); **158**, 366*, 386–8; **180**, 158*. See also *Cryptococcus gracilioides.* (The organism studied by Macleod & Dowling, **161** (XL, 139–48, 1928) and **157**, 1317 was not *P. ovale*, vide Dowling **161** (LI, 7, 1939).)

Torulopsis neoformans (Sanf.) Red. Torulosis. (Syn. *Cryptococcus neoformans, Torula histolytica.*) On man: Smith & Crawford, **153** (XXXIII, 291–6*, 1930); **140** (*1938* II, 1154–7*), as *Cryptococcus meningitidis*; **140** (*1942* II, 394); **150** (*1945* II, 715, 716), as *Debaryomyces neoformans*; **150** (*1948* I, 1082).

Trichosporon beigeli (Rabenh.) Vuill. White piedra. On man (hair): Pye-Smith, **143** (XXX, 439–41, 1879); Cheadle & Morris, **140** (*1879* I, 190); Pernet, **161** (XII, 141, 1900); **161** (XXIV, 131–7*, 1912), study of material from British Guiana; **161** (XXXIV, 265, 1922), reprinted, **162** (XXVI, 190, 1923); **206**, 147; **157**, 437–9*.

MONILIACEAE

Alternaria tenuis Nees. Spores as air-borne allergens. Daily incidence of *Alternaria* spores at Cardiff, 1942–3, Hyde & Williams, **28** (XXIX, 78–85, 1946).

Aspergillus flavus Link. On man (otomycosis), Duncan, **150** (*1945* II, 717).

A. fumigatus Fresen. Aspergillosis. On man: lung, Delikat, **140** (*1945* II, 370–1*) and probably **143** (V, 38–41, 1854) and **143** (XLI, 34–7, 1890); sputum, **143** (L, 349–50, 353), **150** (*1945* II, 715); nasal sinuses, **144** (XII, Laryngol. 187–8); vagina, **162** (XXVIII, 246). **140** (*1942*, I, 394); enzymes, **173** (XXI, 36); serology, **28** (XIV, 73). On birds in captivity: flamingo (lung), Owen, **154** (II, 142, 1832), as 'green vegetable mould or *mucor*'; **143** (L, 357); **154** (*1910*, 134), 48 birds, 1 reptile at the London Zoo; **154** (*1926*, 178–9), contaminated grain in relation to; **145** (CXLIX, 247, 251, 255), association with tuberculosis; **150** (*1945* II, 717). Poultry (black-mould pneumonia, brooder pneumonia), **174**, 139–40; **151**, 414–16; **175**, 454–6. On other animals; monkey (spleen), **154** (*1928*, 90); bison (lung), **61** (CLX, 362); horse (lung), **143** (L, 272). Pathogenic for rabbit, **143** (XXXVII, 549), Rothwell, **153** (VII, 34–52, 1901), and review of earlier literature.

A. niger van Tiegh. On man: Cooke, **14** (VI, 127), **73** (Ser. 2, II, 140*, 1885), as *A. nigricans*, causing otomycosis; **8** (III, 298); Deléphine, **143** (XLII, 423–57*, 1891), associated with melano-mycosis of the skin. On birds: **154** (*1912*, 239); **145** (CXLIX, 251, 1930); **164** (XLIX, 680); **154** (*1926*, 178–9), contaminated grain in relation to. Rothwell, **153** (VII, 34, 1901), pathogenic but never fatal for rabbit and guinea-pig; **173** (XXI, 31–54, 1929), enzymes; **28** (XIV, 73), serology.

Blastomyces dermatitidis Gilchrist & Stokes. North American Blastomycosis. On man: Dowling & Ellsworthy, **144** (XIX, Dermat. Sect. 4–10*, 1925), nail fold then systemic; Castellani, **144** (XXI, Sect. Trop. Dis. Parasitol. 449, 1928), mouth (infection contracted in Egypt), as *B. tulanensis*; **161** (LV, 294–6, 1943), leg; **150** (*1945* II, 716), arm (infection contracted abroad); **150** (*1930* II, 1122); **140** (*1942* I, 292).

Corethropsis hominis Vuill. On man (tinea pedis), Fraser, **162** (XLII, 144, 1939).

Fusarium oxysporum Schlecht. ex Fr. Forming a fungal cast of stomach in man (from Africa), Duncan & Murgatroyd, **155** (XXXII, 6–7, 1938), as *Fusarium* sp., since identified as *F. oxysporum*.

Geotrichum pulmoneum (Catt. & Oliva) Basgal. Isolated from human sputum and lung by Bennett, **53** (XV, 282*, 1842), but fungus not named; **216**, 515*; **182**, 224*; as '*Oidium pulmoneum* Bennett', Cattaneo & Oliva, *Archivo Lab. Bot. Crittogram. Univ. Pavia*, V, 126, 1888 [preface dated 1883]; **8** (III, 278); as *Oospora pulmonea* '(Bennett) Saccardo', *Sylloge*, IV, 16, 1886.

G. rotundatum (Cast.) Red. (Syn. *Endomyces rotundus* Castellani, **150** (*1912* II, 1209); *Monilia rotunda* Cast. & Chalm., **205** (ed. 2, 828); *M. rotundata* (Cast.) Cast., **162** (XVII, 307, 1914); *Oidium rotundatum* (Cast.) Cast. & Chalm., **205** (ed. 3, 1095).) On man: tonsils, Castellani, **206** (115, 1928); toes, **162** (80*, 1940), infection probably contracted in India.

G. rotundatum (Cast.) Red. var. **gallicum** Castellani, **162** (81*, 1940), on man (toes), infection probably contracted in France.

G. rugosum (Cast.) C. W. Dodge, **152**, 219. (Syn. *Endomyces rugosa* Castellani, **150** (*1912* II, 1210); *Monilia rugosa* [Cast.] Cast. & Chalm., **205** (ed. 2, 827), *Hemispora rugosa* (Cast.) Cast. & Chalm., **205** (ed. 3, 1108, as 'Castellani, 1910').) On man: sputum, Castellani, Douglas & Thompson, **162** (XXIV, 149*, 1921), causing 'bronchohemisporosis'; **206**, 128; tonsils, **162** (XXVI, 24, 1923), **206**, 117, **162** (XXVIII, 247–8*, 1925), and vagina; **162** (XXXII, 19*, 1929).

Glenospora semoni Chalmers & Archibald. On man (mycetoma pedis in an Indian soldier), Semon, **161** (xxvii, 299–303, 1915).

Hemispora pararugosa Castellani, Douglas & Thompson, **162** (xxiv, 149*, 1921), from human sputum, causing 'bronchohemisporosis'; **206**, 128; Castellani, **162** (xxxii, 19*, 1929), as *H. rugosa* var. *pararugosa*. **152**, 225, as *Mycoderma pararugosa* (Cast., Dougl. [& Thomps.]) C. W. Dodge.

Histoplasma capsulatum Darling. Histoplasmosis. On man: Derry & Card, **140** (*1942* i, 224–7*); Duncan, **140** (*1942* i, 394); a variant form, Duncan, **150** (*1945* ii, 716), **155** (xl, 364).

Penicillium 'glaucum Link'. Spores experimentally inhaled inducing bronchial catarrh in man, Blackley, **213**, 58.

Sporotrichum anglicum Castellani, **162** (xl, 315*, 1937), from human sputum.

S. schencki (Hekt. & Perk.) Matr. Sporotrichosis. (Syn. *S. beurmani*.) On man: Walker & Ritchie, **150** (*1911* ii, 1–5*), first British case; **144** (iv, Dermat. Sect. 113–21*, 1911), infection contracted in Brazil; **161** (xxv, 33–7, 60–4, 1913); **161** (xxix, 270–3*, 1917), boy in Dublin; **161** (xxx, 174, 1918); **140** (*1942* i, 393), **150** (*1945* ii, 716), knee joint; **143** (lii, 259–70*, 1901), cultural study; **161** (xx, 296–303, 1908), and **150** (*1912* ii, 289–96), reviews; **157**, 433–7* (skin); **146**, 43; **147**, 877*; **158**, 389*.

Dermatophytes

Many clinical and doubtful records have been excluded. Reference is made to representative books on dermatology (**156**, **157**, **158**, **159**, **193**, **214**), and on diseases of the hair (**180**, **195**), but these works have not been indexed exhaustively. The complementary reviews of this group by Tate, **188** (iv, 41, 1929) and Gregory, **188** (x, 208, 1935), and the general account by Ramsbottom & Whitfield, **146**, 46, 1931 may also be consulted.

Achorion gallinae (Mégnin) Sab. Fowl Favus ('white comb'). (Syn. *Lophophyton gallinae*.) On fowls: **22** (Leaflet No. 67, 1908); **150** (*1928* i, 656) and **157**, 381, as *Trichophyton rosaceum*; **174**, 138–9; **151**, 414; **175**, 456. This species appears never to have been transferred to *Trichophyton* where it presumably belongs.

Epidermophyton floccosum (Harz) Langeron & Milochev. Epidermophytosis. (Syn. *E. cruris*, *E. inguinale*.) On man: Whitfield, **144** (iv, Dermat. Sect. 111–12, 1911); **161** (xxiii, 375–402, 1911 and **144**, v, Dermat. Sect. 36–62, 1912) ['Whitfield's ointment']; **156**, 54; **140** (*1922* ii, 791), control; **150** (*1923* ii, 1041), **162** (xxviii, 245, 329, 1925), **189** (cxvii, 343, 345, 354, 1926), **206**, 167, 171, **162** (xli, 378, 1938), pruritis ani and vulvae; **150** (*1928* i, 656); Castellani, **140** (*1928* ii, 595) ['Castellani's paint']; **176** (clxxvi, 219, 1928); **157**, 393*, 395, **418** (nails); **147**, 870; viability in detached scales, **162** (xli, 311, 1938); incidence in the navy, **162** (xlii, 144, 1939), in boys' schools, **145** (ccxxvii, 223–5, 1938); treatment, **162** (xxxvii, 353, 1934), **158**, 361, 362, 384*, 393 (and **179**, iii, 166); **150** (*1940* i, 739); cultivation, **177** (iv, 94); **177** (v, 78); **150** (*1945* ii, 717, 718); **177** (v, 100); **178**, Fig. 139 *a*.

Microsporum audouini Gruby. Human Microsporosis. On man: (children), Tilbury Fox, **181**, 57*, 1863; **214**, 461; **182**, 153*; Adamson, **161** (VII, 206–11, 1895), 'Adamson's fringe' (pls. 1, 3 and 4); Colcott Fox & Blaxall, **161** (VIII, 243*, 1896), **143** (XLVIII, 301–3*, 1897); **183**, 19, 73–82, pl. 1; **184**, 140*; **186**, 551; **195**, 50; **193**, 70; **150** (*1928* I, 656); **157**, 376–7*, 398–400* (clinical); **147**, 865*; **158**, 363 (trunk), 364 (scalp), 375*, 392 (and **179** (III, 166, 1945)); **180**, 179; **177** (III, 168); **150** (*1945* II, 717); **192** (*1944–5*, 96*); (adults) **161** (XXI, 90–1 (and **144** (II (1), Derm. Sect. 89–90)); XXIII, 84–5; XXIX, 114; XLV, 242). Incidence in London, **143** (XLVIII, 301, 1897); **186**, 529, 573; in London County Council Schools, **75** (XXVII, 32–6, 1927); in Edinburgh, **186**, 529; **150** (*1932* I, 93–4), of 'tinea capitis', 1912–30; in England, **150** (*1945* II, 346–9); epidemic in Scotland, **187** (V, 66–8, 1947); detection by ultra-violet light, **150** (*1931* I, 791–2), **177** (III, 170); X-ray epilation, **161** (XV, 66–8, 1903; XXII, 46–9, 1910; XL, 440–50, 1938); thallium acetate epilation, **161** (XXXIX, 307, 1927; XLIII, 59–69, 1930); **150** (*1932* I, 8–10); lice, as carriers of, **140** (*1920* I, 981, 1032); infection of hair, Fox & Blaxall, **150** (*1899* II, 1529–32*); experiments with microsporin, **215**, 129, 146; enzymes, **173** (XXI, 31–54, 1929); staining, **189** (LV, 135–7*, 1895); culture, **161** (XXXVI, 204–6), **177** (IV, 94). See also *Trichophyton tonsurans* sensu latu.

M. canis Bodin. Cat and Dog Microsporosis. (Syn. *M. felineum*, *M. lanosum*.) On man: Fox & Blaxall, **161** (VIII, 352–4*, 381–3, 1896), first description of *M. canis* but fungus not named; incidence in London County Council Schools, **75** (XXVII, 32–6, 1927); in England, **150** (*1945* II, 717; *1947* II, 896); infection from cats and dogs, **161** (X, 37*, 1898; XIV, 327, 1902), **190** (XX, 58, 1912), **191** (LXXVIII, 439, 1922; LXXXV, 303, 306, 1927), **150** (*1945* II, 346), **218** (LXXVII, 65); tinea capitis in an adult, **161** (XXVII, 119, 1920; XXIX, 114, 1927); cultivation, **177** (IV, 94); enzymes, **173** (XXI, 31, 1929). **150** (*1928* I, 656); **157**, 378*; **158**, 363, 364, 376*, 392 (and **179**, III, 166); **192** (*1944–5*, 96*); **180**, 179; **178**, Figs. 136, 137 *a*, *b*, 140. On cats and dogs: **161** (X, 37*, 1898), T.S. and V.S. of cat skin; **206**, 58; **151**, 411. See also under man above.

M. domesticum Webb in Macleod & Muende, **158**, 164, 376–7*, 392, on man (tinea capitis in children) and the cat.

M. equinum (Delac. & Bodin) Guég. On man and the horse: Macleod, **150** (*1928* I, 656); **178**, Fig. 137 *c*; **147**, 865; **151**, 411.

M. gypseum (Bodin) Brumpt. On man: Macleod & Muende, **158**, 375, as *M. fulvum* syn. *Achorion gypseum*.

Trichophyton concentricum Blanch. Tinea imbricata. On man (European): infection first noticed when in Arabia, Castellani, **162** (XXXVII, 363–7*, 1934), **144** (XXVI, 121), **161** (XLV, 23–4), **176**, 17 May 1933), as *Endodermophyton indicum*; **206**, 171*; **158**, 389.

T. discoides Sab. Calf ringworm. On man (and calves): Thomas *et al.* **150** (*1945* II, 347), **150** (*1945* II, 717, 718); **177** (IV, 94) cultivation; **140** (*1932* I, 97–9).

T. equinum Gedoelst. On the horse: Macleod, **150** (*1928* I, 656); **151**, 411.

T. flavum Bodin. On man: Macleod & Muende, **158**, 381*, 392; **179** (III, 166), as *T. cerebriforme.*

T. mentagrophytes (Robin) Blanch. (Syn. *T. asteroides, T. gypseum, T. interdigitale, T. niveum, T. pedis.* See also *T. tonsurans* sensu latu.) On man: Neligan, **194** (pl. 16, fig. 14, as *Microsporon mentagrophytes*; pl. 15, fig. 1, sycosis); **182**, 155; **181**, 57*, sycosis; Tilbury Fox, **140** (*1873* II, 141–2*), **214**, 458, sycosis; **8** (II, 309), as *Sporotrichum mentagrophytes*; Colcott Fox & Blaxall, **143** (XLVIII, pl. 11, fig. 2) and on cats, as 'an ectothrix fungus'; Adamson, **161** (XX, 127, 1908), as 'Trichophyton megalosporon ectothrix du cheval à cultures blanches'; **153** (XII, 232–6*, 1908), infection contracted from calves; **176** (CLXXVI, 222, 1928), tinea pedis, as *Trichophyton niveum*; **161** (XLIII, 121, 1931), erythema multiforme; **145** (CCXXVII, 223, 1938), tinea pedis in boys' schools; **162** (XLII, 144, 1938), tinea pedis in the navy; **158**, 362, 363, 378*, 393 (and **179** (III, 166)); **150** (*1940* I, 739); **150** (*1945* II, 717); **177** (V, 76), tinea pedis; **150** (*1945* II, 347, 348), tinea circinata (from contact with calves and cats) and tinea tonsurans; **177** (IV, 94), cultivation; enzymes, **173** (XXI, 31–54, as *Sabouraudites radiolatus*); **28** (XXI, 98), spiral hyphae; **192** (*1944–5*, 96*). On cattle: Muende & Webb, **197** (XXXVI, 987), and growing saprophytically on dung; **217**, 685. See also under man above. On cats: as *Trichophyton felineum*, Gray, **191** (LXXIX, 314, 1923), **151**, 411; as *T. niveum*, **150** (*1928* I, 656). See also under man above. On the dog: Macleod, **150** (*1928* I, 656), as *T. niveum.* On the horse: **217**, 684. On the mouse: Parish & Craddock, **196** (XII, 209, 1931), ringworm epizootic which spread to four laboratory attendants.

T. ochraceum Sab. On man: **157**, 382; **180**, 185, beard.

T. persicolor Sab. On man: Adamson (vide **198**, 371), as 'the peach-coloured trichophyton'; **157**, 381; **158**, 379*, as *T. purpureum*; **150** (*1945* II, 717); **177** (IV, 94), cultivation.

T. plicatile Sab. On man: Sequeira, **161** (XVIII, 269, 1906; XXIV, 207–15*, 1912) and **159**, 404, trichophytic granulomata of the umbilicus; **157**, 415, tinea barbae; **159**, 392. [This species is probably *T. epilans* Mégnin.].

T. quinckeanum (Zopf) Macleod & Muende. Mouse favus. (Syn. *Achorion quinckeanum.*) On man (and the mouse): Roberts (**185**, 22–3), the 'achorion of Quincke' said to be seldom met with in Britain; Adamson, **144** (II, Dermat. Sect. 1–3*, 1909 and **161** (XX, 365)), **161** (XXI, 116–17), **144** (II, Dermat. Sect. 91, 103–4*), mouse and child; **161** (XXV, 138, 165, 1913); **161** (XXVI, 84 (child), 323 (mouse), 1914), **161** (XXXVI, 168, 1924); **150** (*1929* II, 1194*); **157**, 425*; **147**, 869*; **158**, 363, 383*, 393 (and **179**, III, 166); **150** (*1945* II, 717); **177** (IV, 94); **159**, 405*.

T. roseum Bodin. On man: beard: Adamson, **161** (XX, 258, 1908), as 'Trichophyton ectothrix à cultures roses' (= *T. rosaceum* vide **198**, 258); **144** (II, Dermat. Sect. 83–4, 161, 1909); **161** (XXI, 325, 1909); Bolam, **161** (XXIV, 1–13*, 1912), general account and 16 cases of tinea barbae and tinea circinata; **156**, 58; **157**, 381*; **147**, 866*; nails, **171** (N.S. VI, 125*, 1911), **159**, 404; feet, **150** (*1945* II, 717); **177** (IV, 94, 1945), cultivation. [This species is probably *T. megnini* Blanch.]

T. rubrum (Cast.) Sab. (Syn. *Epidermophyton rubrum.*) On man: Semon, **161** (XXXIV, 355, 397–400*, 1922), nails; Castellani, **150** (*1923* II, 1041), **189**, (CXVII, 343, 345, 1926), **162** (XLI, 378, 1938), pruritus ani; tinea pedis, **162** (XLII, 144, 1939), in the navy, **150** (*1945* II, 717, 718), **177** (V, 78, 1946); **177** (IV, 94, 1945), cultivation; **147**, 870*; **158**, 362 (groin), 379*, 393 (and **179**, III, 166, 1945), as *Trichophyton purpureum.*

T. sabouraudi Blanch. (Syn. *T. acuminatum.*) On man: Colcott Fox, **144** (II, Dermat. Sect. 58* and **161**, XXI, 283, 1909); **171** (N.S. VI, 125*, 1911); experiments with trichophytin, **215**, 130; **75** (XXVII, 32–6, 1927), incidence in London County Council Schools; **150** (*1928* I, 656); **157**, 380*, 415, tinea barbae; **158**, 364, 380*, 392 (and **179** (III, 166, 1945)); **150** (*1945* II, 349, 717, 718), hair and nails; **177** (IV, 94, 1945), cultivation.

T. schoenleini (Leb.) Langeron & Milochev. Favus. (Syn. *Achorion schoenleini.*) On man: Bennett, **200** (II, 504–19*, 1842) and **53** (XV, 277*, 1842), the fungus nature of favus cups described but fungus not named; **194** (pl. 16, fig. 5; pl. 15, figs. 2 and 3); **182**, 162; **181**, 56, pl. 1, fig. 1); **201**, 50, 56; **214**, 429; as *Oidium porriginis*, **19**, no. 546, **15**, 604, **18**, 350; as *Oospora porriginis*, **8** (III, 278); as *Achorion vulgaris*, **185**, 11, 19; **161** (III, 101–5*, 1891); **184**, 139, fig. 16; **195**, 60; **193**, 92; **171** (N.S. VI, 126, 1911), nails; **156**, 60–2*; **75** (XXVII, 32–6, 1927), incidence in London County Council Schools; **215**, 131, experiments with favin; **173** (XXI, 31–54, 1929), enzymes; **150** (*1931* I, 792), detection by ultraviolet light; **161** (XLIV, 500, 1932; LIV, 237, 1942); **157**, 421–9*, **147**, 869*; **158**, 363 (trunk), 364 (scalp), 383*, 393 (and **179** (III, 166, 1945)); **180**, 190; **150** (*1945* II, 717, 718); **192** (*1944–5*, 96*); **177** (IV, 94, 1945), cultivation; **178**, fig. 133, 139 *a*.

T. sulphureum Colcott Fox. On man: Colcott Fox, **144** (II, Dermat. Sect. 57*, as 'the primrose crater'), reprinted, **161** (XXI, 280*, 1909, as *T. sulphureum*), hair; Low, **171** (N.S. VI, 125*, 1911), nails (as *T. crateriforme flavum*); **161** (XXVI, 431, 1914), tinea circinata; **215**, 130, experiments with trichophytin (as *T. crateriforme flavum*); **75** (XXVII, 32–6, 1927), incidence in London County Council Schools; **150** (*1928* I, 656); **157**, 380*; **147**, 866*, (as *T. crateriforme flavum*), causing ringworm of the scalp in Scotland; **158**, 380*, 392 (and **179**, III, 166, 1945); **150** (*1945* II, 717).

T. tonsurans Malmst. (Syn. *T. crateriforme.*) On man: Fox & Blaxall, **143** (XLVIII, pl. 13, fig. 1), Fox, **144** (II, Dermat. Sect. 55* (and **161**, XXI, 279*), 1909); **75** (XXVII, 32–6, 1927), incidence in London County Council Schools; **173** (XXI, 31–51, 1929), enzymes; **150** (*1928* I, 656); **157**, 380*, 415 (beard), 418 (nails); **158**, 364, 380*, 392 (and **179**, III, 166, 1945); **150** (*1945* II, 347 (tinea circinata), 717 (and tinea capitis)); **177** (IV, 94, 1945), cultivation.

During the latter half of the nineteenth century (and occasionally since) *T. tonsurans* was frequently used in a wide sense to cover all species of *Trichophyton* or even all ringworm fungi. The following references have a historical interest: **182**, 140*; **181**, 56, pl. 1, fig. 2;

201, 20, 56; **214**, 432; Thin, **202**, **163** (LXI, 179–88, 1878), demonstration of fungus in skin of horse, **67** (XXXIII, 234–46, 1881), experiments in artificial cultivation; Taylor, **163** (LXII, 177–88, 1879), T.S. and L.S. of infected human hairs; Roberts, **161** (I, 359–65, 1889), artificial cultivation, **185**, 10, 15*, *T. capitis vulgaris* n.nom. for *T. tonsurans* in the wide sense; **22** (Leaflet No. 95, 1908), on cattle.

T. tenuishypha Castellani, **162** (XLII, 376, 1939). On man (hand, arm, trunk), London.

T. violaceum Bodin. On man: Colcott Fox, **144** (II, Dermat. Sect. 62* (and **161**, XXI, 285*), 1909); **156**, 58 (beard); **75** (XXVII, 32–6, 1927), incidence in London County Council Schools; **150** (*1928* I, 656), beard and scalp; **157**, 382*, 415 (beard), 418 (nails); **158**, 364, 381*, 393 (and **179**, III, 166, 1945).

BACTERIA (SCHIZOMYCETES)

ACTINOMYCETALES

Actinomyces gibsoni C. W. Dodge, 1935 (August) (**152**, 722). Isolated from enlarged human spleen, Gibson, **153** (XXIII, 357–8, 1920), but not named. Cultural characters described and tentatively designated '*A. gibsonii*', Erikson, **145** (no. *203*, 36, 9, 15, Sept. 1935). [Aerobic. *Streptomyces gibsonii*.]

A. graminis Topley & Wilson, **148** (ed. 1, pp. 242, 250, 1929), the aerobic *A. bovis* of Bostroem, is a saprophyte of grains and grasses sometimes found associated with man and animals. *Streptothrix bovis communis* Foulerton, **143** (LIII, 87, 95) and perhaps other records included under *Actinomyces israeli* (q.v.) probably belong here.

A. israeli (Kruse) Lachn.-Sand. Actinomycosis. [Actinomycosis in man and cattle is frequently attributed to *A. bovis* Harz, but because this binomial is a nomen confusum (see Erikson, **145** (no. *240*, 46)), it is preferable to designate the cause of actinomycosis in man *A. israeli*. From a study of Australian material, Erikson (loc. cit.) concluded that actinomycosis in cattle is caused by a distinct but allied species. Here the records for anaerobic forms from man and animals have been united under *A. israeli*. See also *A. graminis*.] On man: Shattock, **143** (XXXVI, 254–61, 1885) appears to be the first published British record (liver, diagnosed post-mortem), but Acland, **143** (XXXVII, 546–9, 1886) claimed to have first recognized actinomycosis in this country, early in 1885. Powell *et al.* **163** (LXXII, 175–208, 1889) were the first in Britain to diagnose pulmonary actinomycosis during life. **143** (XL, 408–43), as *A. hominis*; **163** (LXXV, 63–84*), appendix; **163** (LXXV, 85–102*), urinary and alimentary tracts; **163** (LXXV, 103–7), face and neck; Foulerton, **140** (*1899* II, 779), as *Streptothrix actinomycotica*, **149** (XIV, 50, 53*), as *S. bovis communis* [n.nom. for *Actinomyces bovis*], **140** (*1905* I, 1200), **142**, 308, as *Streptothrix hominis* III [*Actinomyces bovis* vide Chalm. & Christoph. **141** (X, 256)], **140** (*1910* I, 553*, 626–7), as '*Streptothrix hominis* IV'; **153** (XIV, 164–73), central nervous system; **165** (I, 197–212); **150** (*1920* I, 435), cultivation; **161** (XLVI, 12–19), skin, *Bacillus actinomycetum comitans* in relation to (see also **150** (*1921* I, 893); **146**, 85–6); **157**, 429–33*, skin; **166**, 16–24*, biological characters; **158**, 352, skin; Erikson, **145** (no. *240*), taxonomy; **150** (*1945* II, 715, 716, 717), central nervous system, lung, appendix; **140** (*1948* I, 439), liver with recovery. Treatment with vaccine (actinomycotin), **150** (*1908* I, 554–7), **150** (*1913* I, 991–2), **150** (*1916* II, 488); sulphonamides, **140** (*1940* II, 707), **150** (*1943* II, 106); penicillin, **150** (*1945* II, 728). Historical and general surveys, **150** (*1889*, 1339–44); **140** (*1897* I, 11–18); **142**, 324–43; **146**, 78–85; **166**; **147**, 473*; **148** (ed. 3, 379, 382–3, 1270–4). On cattle: MacFadyean, **150** (*1889*, 1339–44); **140** (*1897* I, 11–18); **149** (XX, 189), incidence in Britain; **150** (*1922* II, 1163–4), vaccine treatment; **149** (XXXVI, I, 1923), as *Streptothrix israeli*; **217**, 143; **151**, 311–16*. **75** (XV, 195–207), confusion with *Actinobacillus* (see **146**, 87); **164** (N.S. XII, 55–9; XV, 15–17), 'actinomycotic' mastitis caused by *Staphylococcus pyogenes*. On the cat: Edington, **164** (N.S. XIV, 311–12, 1934). On the pig: Davies & Torrance, **149** (XLIII, 220), as *Streptothrix actinomyces*.

A. muris-ratti (Schottmüller) Topley & Wilson, **148** (ed. 1, 251, 1929). On the rat (nasopharynx): Strangeways, **153** (XXXVII, 45–51, 1933), as *Streptobacillus moniliformis*. *Actinomyces muris* Topley & Wilson, **148** (ed. 2, 251, 1936) n.nom. for *Streptothrix muris-ratti*; ibid. ed. 3*, 285, 1276–8. [Aerobic.]

Micromonospora fusca Jensen. Isolated by Duncan from diseased hair, Erikson **145** (no. *240*, 28).

Nocardia appendicis Chalmers & Christopherson, 1916 (**141**, X, 256), n.nom. for *Streptothrix hominis* IV, Foulerton (**140**, *1906*, I, 970), from human appendix. Foulerton, **142**, 308; **140** (*1910* I, 553*, 626–7, as '*S. hominis* III').

N. asteroides (Eppinger) Blanchard. (Syn. *Actinomyces asteroides*, *Streptothrix eppingeri*.) On man: Foulerton & Jones, **143** (LIII, 87, 97, 1902); **142**, 308, **140** (*1910* I, 551, 629*, 769); **144** (VI, Surg. Sect. 138) and **140** (*1913* I, 382); **146** (VIII, 75); **145** (no. *203*, 10, 26); **147**, 477; **148** (ed. 3, 388).

N. caprae (Silberschmidt) Emmons. (Syn. *Actinomyces caprae* (Silberschmidt) Ford.) On man (spleen in two cases (brother and sister) of acholuric jaundice), Gibson *et al.* **150** (*1938* I, 612–14); **149** (XIV, 56*), **143** (LIII, 87, 99), **142**, 308, **140** (*1910* I, 556), as *Streptothrix caprae*; **145** (no. *203*, 37, 26), cultural characters; **148** (ed. 3, 387). On the horse (submaxillary abscess), Dean, **143** (LI, 26–47, 1900); organism not named but *Actinomyces caprae* group vide **148** (ed. 3, 1276).

N. foulertoni Chalmers & Christopherson, (**141**, X, 256, 1916), n.nom. for *Streptothrix hominis* Foulerton in Foulerton & Jones (**143**, LIII, 79, 87, 100, 1902), pulmonary infection in a woman (case first described but organism not named by Foulerton, **140** (*1899* II, 779). As *S. hominis* I, Foulerton **140** (*1905* I, 1200; *1906* I, 970), **142**, 309, **140** (*1910* I, 553*, 626–7).

N. leishmani Chalmers & Christopherson (**141**, X, 255, 1916), based on *Streptothrix* sp. isolated by Birt & Leishman (**75**, II, 120–8) from a case of fatal lung disease and pericarditis in man, **145** (no. *203*, 10, 27, 37).

N. londoniensis Chalmers & Christopherson (**141**, x, 256, 1916), n.nom. for *Streptothrix hominis* II, Foulerton, **140** (*1905* I, 1200), from tongue abscess in man. Foulerton, **140** (*1906* I, 970; *1910* I, 553*, 626–7), **142**, 309.

N. luteola (Foulerton) Chalmers & Christopherson (**141**, x, 265, 1916). *Streptothrix luteola* Foulerton, in Foulerton & Jones (**143**, LIII, 75, 86, 93, 1902), from a case of conjunctivitis in man. Foulerton, **140** (*1905* I, 1200 (alveolar abscess of the mandible); *1906* I, 970), **142**, 304*.

N. macropodidarum Fox. On Bennett's wallaby (*Macropus bennetti*), Zoological Society of London, Scott **154** (*1925*, 799–814).

N. madurae (Vincent) Blanch. Mycetoma (Madura Foot). On man (Indian): infection contracted in Aden, Duncan *et al.* **155** (XXXII, 427–9, 1939), as *Actinomyces madurae*. **143** (LIII, 87, 96, 1902), as *Streptothrix madurae*; **142**, 303; **140** (*1910* I, 551); **146**, 74; **148** (ed. 3, 383–4, 1275).

N. minutissima (Burch. & Barens.) Verdun. Erythrasma. (Syn. *Microsporon minutissima*.) On man: Whitfield, **156**, 72*; **157**, 439–40*; **158**, 352*, 363; **159**, 407–8*.

N. splenica Gibson, **160**, 130*, 1930, from human spleen. Erikson, **145** (no. *203*, 36, 1935; cultures 4575, 450, 4579, 4581, 4583), tentatively based five new species on Gibson's cultures of *N. splenica*.

N. tenuis Cast. Trichonocardiosis. (Syn. *N. tenuis* Castellani, 1911 (**161**, XXIII, 341); *Discomyces tenuis* [Cast.] Castellani, 1912 (**144**, VI, Derm. Sect. 23–7); *Actinomyces tenuis* (Cast.) C. W. Dodge, 1935 (**152**, 715).) On man (hair). During the summer of 1920–1 over 80% of the patients admitted to the Tropical Section of Orpington Hospital infected, vide Castellani & Wilkinson, **161** (XXXIV, 255–6, 1922; reprinted, **162**, XXVI, 186–90), who distinguished trichomycosis axillaris flava, nigra, and rubra (see also **144**, XVI, 97, and **161**, XLIII, 88; **206**, 140) caused by *Nocardia tenuis* and *N. tenuis* in association with a black pigment-producing coccus (*Micrococcus nigrescens* Cast.) or a red pigment-producing coccus (*M. castellani* Chalm. & O'Farrell), respectively. **162** (XXIX, 222); **157**, 443–5*; **158**, 352.

DOUBTFUL AND EXCLUDED SPECIES

Cercosporella vexans Massee in Russ, **140** (*1923* I, 77*), from lesions on palmar surfaces of the forearms of factory workers handling foodstuffs. Listed by Dodge (**152**, 505) as a doubtful synonym of *Ectotrichophyton mentagrophytes* var. *radiolatum* [*Trichophyton mentagrophytes*].

Lorum uteri Wilkinson, **140** (*1849*, 451), from the human uterus. **216**, 367; **182**, 133*, as *Leptomitus* (?) *muci uteri*; **181**, 65, as *Leptomitus uteri*.

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  - S. schencki
  - Torulopsis neoformans
  - Trichophyton concentricum
  - T. discoides
  - T. flavum
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  - T. ochraceum
  - T. persicolor
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- MOUSE
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NOTES ON *CIRCINELLA SIMPLEX* VAN TIEGHEM

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(With 2 Text-figures)

In the spring of 1946 a sample of soil was received in the Department of Mycology, University of Edinburgh. It had been obtained from a castle in Perthshire, Scotland, where a drain in the kitchen regions had become choked, and the soil around it was covered with a slimy mass of fats on which fungi were growing. Cultures were made from this material, and amongst the fungi obtained was *Circinella simplex* van Tieghem.

C. simplex appears to have been isolated only twice before—first by van Tieghem (1875) from dog excreta in France, and secondly by Zycha (1935) who obtained it from a soil sample from Brazil. Niethammer (1937) and Gilman (1945) both quote these records without adding to them. Zycha states that the height of the aerial mycelium above the substratum (2–3 mm.) is a constant character which appears to separate it from *C. spinosa*, where it is 20 mm. high. This is really a quotation from van Tieghem's paper (1875, p. 93). In the very next part of the sentence, Zycha adds that this character varies strongly, and that he believes that the two species are identical. The strain isolated from Perthshire has retained its low growth habit through ten subcultures (twenty months) on malt-extract agar, which justifies van Tieghem's separation of these two species.

The figures given by van Tieghem (1875) for *C. simplex* show a single sporangium with a sterile spine, in addition to five sporangiospores and four sporangiophores on a much smaller scale. The complete absence of sterile spines from the fungus from Perthshire made identification somewhat doubtful at first. However, reference to van Tieghem and Le Monnier's paper (1873), in which they first describe the genus, shows that in some of the figures of *C. spinosa* the spines are absent. In their description they do not mention the spines as a special character of the genus, and Zycha (1935) suggests that spine production appears to be greatly influenced by external conditions. It would, therefore, appear that they may or may not be present.

In culture on 2½% malt-extract agar, the aerial mycelium is some 2–3 mm. high and of a fawn colour; buffy citrine according to the Ridgway (1912) colour standards (Pl. 16, colour 19'k). The sporangiophores are of the same colour and are regularly sympodially branched, bearing up to twenty or more fawn-coloured sporangia on short curved branches. According to van Tieghem (1875), the number of sporangia produced is up to fifteen, or even twenty, but in the present material over twenty has been the rule rather than the exception (Fig. 1). On occasions the sporangiophores were even branched dichotomously (a condition figured by van Tieghem and Le Monnier (1873) for *C. spinosa*), giving rise to two branches bearing some seven or more sporangia each.

At first a young colony on malt-extract agar resembles a culture of *Mucor*, because of the number of auxiliary sporangia produced, but after three days a small branch can be seen two-thirds of the way up the stronger sporangiophores towards the edge of the culture, and in due course a large sporangium is formed at the end of this branch. Mounting such a young preparation in lactic acid and cotton blue reveals that prior to the formation of the branch, a cross wall is laid down, cutting off the mature first-formed sporangium from the mycelium. On rare occasions, in older cultures (two to three weeks old), some of the auxiliary sporangia may be carried to a height of 5 mm. above the substratum, but they seem to be confined to the immediate area of inoculation.

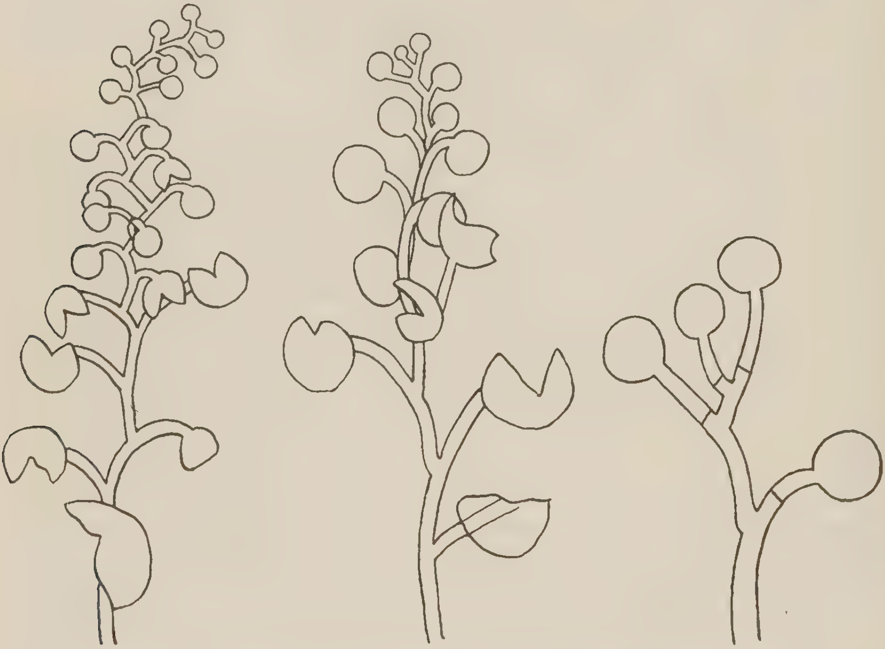

Fig. 1. Sporangiophores and sporangia of *Circinella simplex* v. Tiegh.

On mature sporangiophores, the sporangia range in diameter from 3–39μ (Zycha 25–50μ), but as the sporangial wall is very brittle, it is difficult to measure the larger sporangia with accuracy. The wall of the sporangium is thickly encrusted with crystals of calcium oxalate.

Sporangia decrease in size as one succeeds another up the sporangiophore. The question naturally arises, if this decrease is regular and gradual, or if it is irregular, and then due to a nutritional factor. Successive sporangia on mature sporangiophores were measured from the base upwards, and gave the following curves when the decrease in diameter in μ of successive sporangia upwards is plotted against unity. In all six series of measurements shown, the readings conclude with the terminal sporangium, and all of them are taken from twelve-day-old Petri dish cultures.

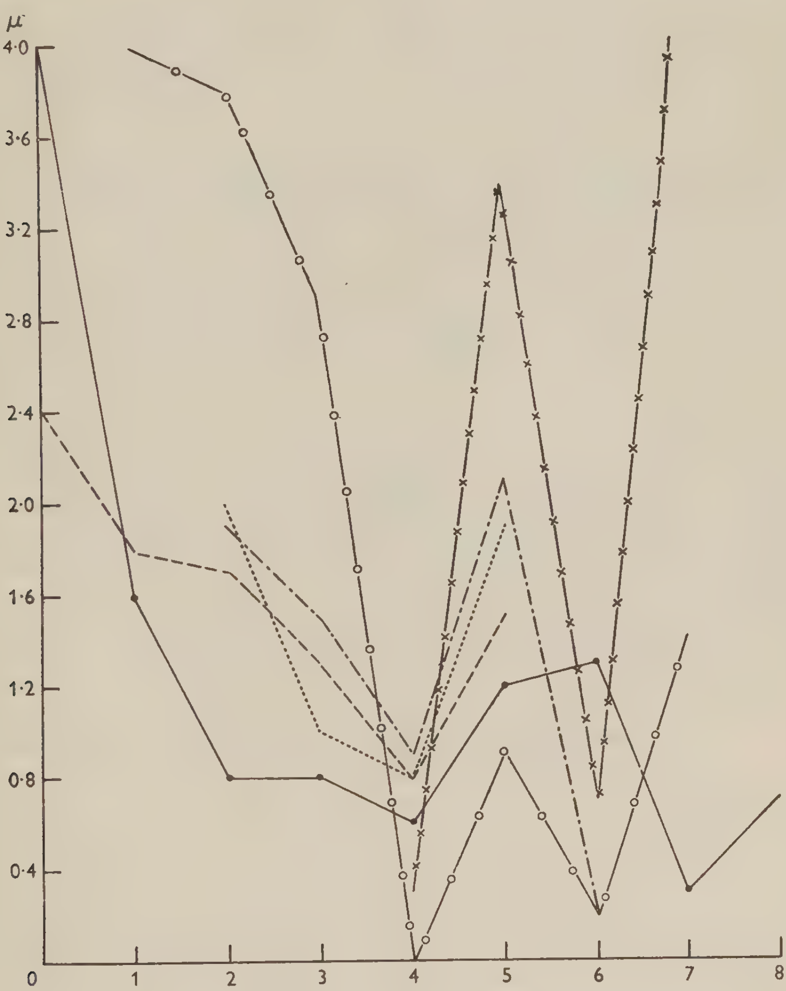

Fig. 2. For description see text.

These six sets of readings show basically the same pattern, the presence of two minima, that is to say, there are two stages during development when successive sporangia on the same sporangiophore are practically identical in diameter. There is a remarkable similarity in the form of these curves, but as can be seen, they do not all begin at the same point. That is to say, the minima do not always occur at the same point on each sporangiophore, though the interval between the two minima is constant. That these minima do not always occur at the same point is due to the growth of the fungus. Sporangiophores near the edge of the culture are younger than those towards the centre, and as the interval between the two minima is constant, it shows clearly that the main factor governing sporangial size is a nutritional one, with the depth of the medium, and consequent amount of nutriment as the limiting factor.

In a three-day culture on 2½% malt-extract agar, the largest auxiliary sporangium was 19·8μ and the smallest 3·2μ, with the average of 12·9μ. The smallest sporangium contained five spores, though only 3·2μ in diameter.

In a sporangium of diameter 25μ measured from the tip of its junction with the sporangiophore, the columella was 13μ when measured in the same way. In a sporangium of 18μ, the columella was 10μ measured in the same way.

The spores are hyaline, oval to irregularly oval, 2–4μ in size (Zycha 3–5μ), though an occasional giant spore up to 8μ has been seen.

This fungus is said to be heterothallic. No zygospores have been seen. Giant cells have not been observed.

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AQUATIC HYPHOMYCETES FROM SWITZERLAND

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(With 3 Text-figures)

Working in Britain I found a special flora of aquatic Hyphomycetes growing on submerged decaying leaves of broad-leaved trees and shrubs in streams and other well-aerated water. Some twenty-five species of these fungi were recognized. Six had previously been described, though rather imperfectly, but their special relationship to submerged leaves had not been appreciated.

My early work (Ingold, 1942) was limited to a small stream in Leicestershire, but later, scattered observations (Ingold, 1943) covering England, Wales and Ireland, showed that this flora was widespread, and suitable decaying leaves from any stream never failed to give crops of these fungi.

The examination of leaves from other localities in the British Isles added only a few to the list of species reported from the stream in Leicestershire, and, although intensive search would almost certainly add a few to the list of species already known, it is doubtful if many more await discovery.

It was clearly of interest to know if the same flora was to be found elsewhere in Europe and, on a short visit to Switzerland in the late summer of 1947, I took the opportunity to study the fungi of submerged decaying leaves of willow and alder in alpine waters. My observations were made possible by the kindness of Prof. E. Gäumann of Zürich who not only lent me a microscope and other necessary apparatus, but also suggested an excellent locality (Surlej-Silvaplana) for my operations.

Surlej (1800 m.) lies in that valley of the Upper Engadine which includes a series of lakes (St Moritzersee, See von Campfer, Silvaplanersee and Silbersee) linked by short stretches of river. Bordering these stretches, on either side of the main channel, are patches of willow scrub, flooded doubtless when the river is in spate, but through which in summer there trickle occasional small streams. Willow leaves from these small streams were examined in the region between St Moritzbad and the See von Compfer. In addition to the main river, there are numerous little torrents cascading down the mountain sides to join the water system of the valley. The steep ides of the valley up to about 2100 m. bear a fine coniferous forest, but bordering the torrents are alders and willows. Decaying leaves of these, caught between the small boulders of the streams, were also examined.

It was at once apparent that the decaying leaves, both from the main tream and from the tributary torrents, bore rich crops of aquatic Hyphomycetes.

Altogether six collections, each of 10–20 leaves, were carefully examined. These yielded nine known species (Table 1 and fig. 1) and, in addition, an interesting new fungus apparently referable to the genus *Anguillospora.* All these species figured in more than one collection.

Table 1

Species	Notes
Alatospora acuminata Ingold	Very abundant
Lemonniera aquatica de Wild.	Very abundant
Flagellospora curvula Ingold	Very abundant
Tetracladium marchalianum de Wild.	Very abundant
Articulospora tetracladia Ingold	Abundant
Tricladium splendens Ingold	Seen three times
Heliscus aquaticus Ingold	Seen twice
Tetracladium setigerum (Grove) Ingold	Seen twice
Tricladium angulatum Ingold	Seen twice

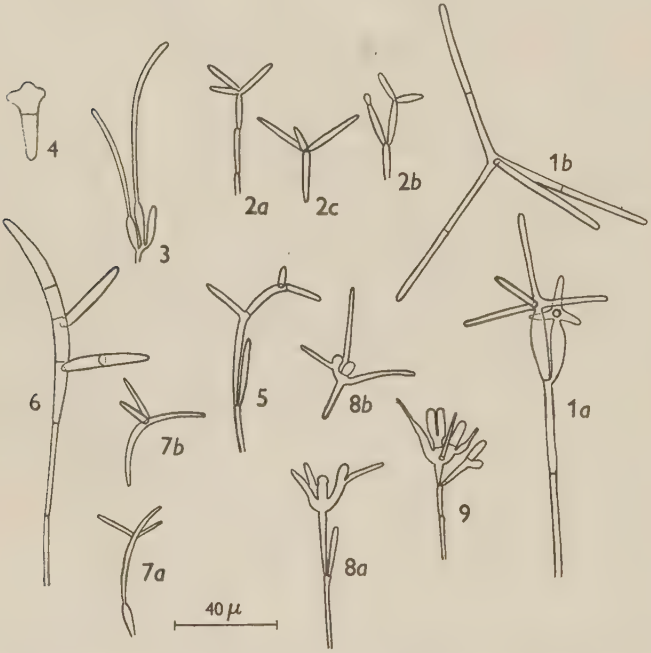

Fig. 1. Aquatic Hyphomycetes drawn with the aid of a camera lucida from specimens collected in Upper Engadine. 1, *Lemonniera aquatica*, *a*, conidiophore with two developing spores; *b*, spore. 2, *Articulospora tetracladia*, *a*, conidiophore with one ripe spore; *b*, conidiophore with two developing spores; *c*, spore. 3, *Flagellospora curvula*, conidiophore bearing two spores. 4, *Heliscus aquaticus*, spore. 5, *Tricladium angulatum*, conidiophore with one ripe spore and an unbranched spore-primordium. 6, *Tricladium splendens*, conidiophore with ripe spore. 7, *Alatospora acuminata*, *a*, conidiophore with ripe spore; *b*, liberated spore. 8, *Tetracladium marchalianum*, *a*, conidiophore bearing one nearly mature spore and an unbranched spore-primordium; *b*, spore. 9, *Tetracladium setigerum*, conidiophore bearing one mature spore and a spore-primordium developing its first lateral branch.

These organisms clearly formed the characteristic fungus flora of the decaying leaves and, although a few pythiaceous fungi were seen, they were few and far between. Submerged decaying leaves of herbaceous plants and of conifers were examined, but no trace of aquatic Hyphomycetes was found on them.

With the exception of *Tetracladium marchalianum* these fungi are recorded for the first time from Switzerland. De Wildeman (1894) reported *T. marchalianum* from a pond in Geneva. However, even the restricted conception of that species which he proposed in 1894 included at least two species (see Ingold, 1942) and, as he gave no figures of the Geneva material, it is not possible to say what species he actually observed.

A noteworthy feature of the list (Table 1) is the absence of *Clavariopsis aquatica* and *Anguillospora longissima* which are almost invariably present on submerged decaying leaves from streams in Britain.

Anguillospora flagellifera n.sp.

This fungus (Figs. 2 and 3) was found in the first instance on a submerged leaf decayed too far for certain identification, but it was later collected on a decaying willow leaf from another stream.

The mycelium is branched, septate, hyaline and ramifies in the tissue of the leaf, from which the conidiophores grow out at right angles. The conidiophore is simple, narrow (2μ diam.) towards its base, but inflated near its apex (6–9μ diam.). The single elongated, hyaline conidium (aleuriospore) is terminal. It is 140–200μ long, truncate at its base where the diameter is 6–8μ, but it widens upwards becoming 10–14μ broad at a distance of 40–50μ from the base, but above that it narrows, to form a whip-like terminal part only 2–3μ in diameter. This whip-like end is curved in such a way that the spore does not lie in a single plane. The conidium has 4–6 septa and is very pearly in appearance no doubt due to to a reserve of glycogen. When mature the spore separates from its conidiophore apparently by the breakdown of the middle lamella at the junction between the two. Germination is by a single, very straight germ tube developed laterally from near the base of the lowermost cell of the spore, quite often before it is shed. The liberation of the spore does not necessarily bring the activity of the conidiophore to an end, for it may grow out laterally, below the scar left by the separation of the first spore, and give rise to a second.

The reference of this well-marked species to the genus *Anguillospora* seems justified because it is an aquatic Hyphomycete with a worm-like spore of the aleuriospore type borne singly on a conidiophore which is usually simple. It differs from the only other species, *A. longissima*, in a number of ways. First, the spore is wider and its two ends are strikingly different, whilst in *A. longissima* it is almost impossible to distinguish base from apex in the liberated conidium. Secondly, the conidium germinates by a single lateral germ-tube arising from the basal cell, but that of *A. longissima* germinates by basal and apical tubes in line with the longitudinal axis of the spore. Thirdly, the conidiophore with its inflated apex contrasts with that of *A. longissima*, for in that species it is difficult to be sure, until the spore is about to be shed, where the conidiophore ends and the conidium begins. Fourthly, no separating cell, such as occurs in *A. longissima*, is developed.

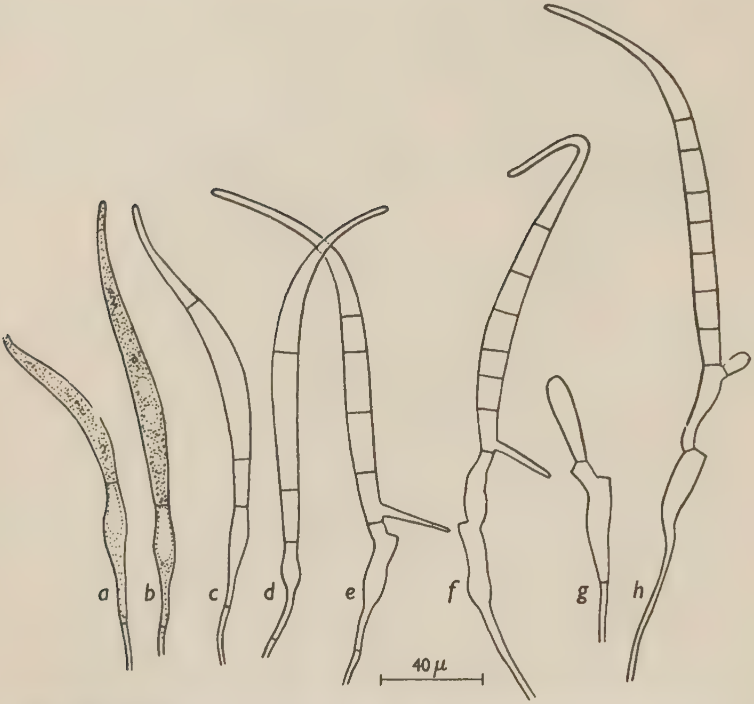

Fig. 2. *Anguillospora flagellifera*. Conidiophores with conidia. Contents shown only in *a* and *b*. In *e* and *f* the conidium has germinated while still attached. In *e*, *f*, *g* and *h* scars left by detached conidia are shown. In *h* a very young spore-primordium is shown to the right of a mature spore.

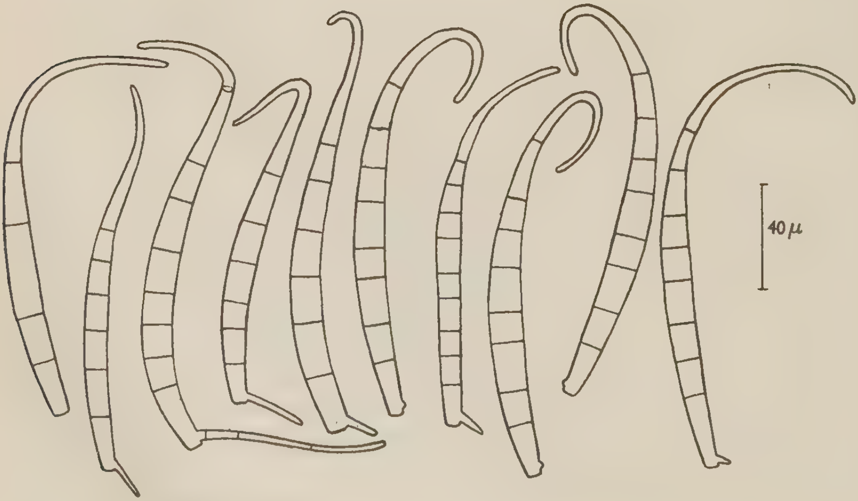

Fig. 3. *Anguillospora flagellifera*. Conidia, most of which have started to germinate.

Anguillospora flagellifera sp.nov.

Fungus submersus aquaticus, mycelio repente, hyalino, septato, ramoso. Conidiophora simplex, basim versus 2μ lata, summa parte inflata 6–9μ lata. Conidium (aleuriospora) acrogenum, hyalinum, 4–6 septatum, vermiforme vel falcatum, 140–200μ longum, ad basim truncatum 6–8μ latum, in medio 10–14μ latum, ad apicem filiforme curvatum et ad 2–3μ diam. attenuatum. In germinatione oritur una lateralis hypha solum e conidii infima cellula.

Hab. In foliis putrescentibus in flumine submersis, in Valle Engadin Helvetiae.

SUMMARY

Aquatic Hyphomycetes form the characteristic fungus flora of submerged decaying leaves of alder and willow in Switzerland as in Britain. Leaves collected from streams in the Upper Engadine in August 1947 yielded nine species already reported from Britain and, in addition, a species, *Anguillospora flagellifera*, apparently new to science.

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Note. Since this paper was accepted, I have found *Anguillospora flagellifera* on a decaying leaf from a small lake at Clandeboye, Co. Down, Ireland, during the Autumn Foray, 1948.

REVIEW

The Fungi. By F. A. Wolf and F. T. Wolf. New York: John Wiley and Sons; London: Chapman and Hall. 1947. Vol. I, x+438 pp. 153 t.f., 36*s.* net; Vol. II, xii+538 pp., 82 t.f., 39*s.* net.

The first volume of *The Fungi* describes the morphology, development and classification of the fungi, the second treats of the biology and physiology of the living fungus, as well as of such general matters as geographical distribution and the fossil record. In intention, the work is a comprehensive account of what is known about fungi. It should be useful to the working mycologist, for there is little of mycological interest which is not presented, either directly, or by reference to other works. But the reader will need to verify many of the statements which appear in the two volumes.

The authors make it clear that they are mainly interested in the living fungus, and that they consider theoretical developments to be of secondary importance. Perhaps the most striking feature of the work is the introduction of free comments on what may be called botanical politics. It may well be that some readers will regard these comments as immature, or indiscreet, or even as unnecessary. But they have their value, and one chapter (Vol. II, chapter 18), which consists largely of comments, merits special mention. The chapter contains little which has not been said often in private conversation or in public discussion, and little or nothing which will be new to the thoughtful reader, yet it is of value as a careful exposition of matters which are too commonly disregarded.

Of the two volumes, the second appears likely to be the more useful. It contains a stimulating account of the fungus in action. Such an account, in so convenient a compass, does not seem to have been attempted since 1890, when Zopf's *Die Pilze* appeared (in Schenk's *Handbuch der Botanik*, Vol. IV, pp. 271–755). Now, when mycology is developing so rapidly and when the biology and physiology of the fungi come more and more into prominence, this second volume will soon be in need of revision. The authors will benefit their readers by producing a second edition in which additional information will be included, and in which the misprints and mis-statements to be found in the existing volume will have been corrected. It is disappointing to have to grumble at so forward-looking a work as *The Fungi*, but there are regrettable misprints. For instance (Vol. II, p. 87), *Penicillium citrinum* gives citrinin, not penicillin, and flavicin is active against Gram-positive bacteria. The chemical formulae and equations are not always correctly printed, and it is surprising to find *Amanita muscaria* figured as *A. phalloides* (Vol. II, fig. 58) and to find *A. caesarea* included as a common poisonous fungus. Those two troublesome words, 'substratum' and 'synonymy' seem to have defeated the proof readers, and the indexes are not wholly reliable.

Even in these days, £3. 15*s.* 0*d.* for the two volumes is a heavy price. Many students in this country will not be able to afford to buy the books. Maybe, if a revised edition appears, it will not only be more accurate in its facts, but also priced at a level nearer to that possible to the student's purchasing power.

B. BARNES

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REVISED LIST OF BRITISH AGARICS AND BOLETI

A. A. PEARSON & R. W. G. DENNIS

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